STUDIES ON THE EARLY STAGES OF DEVELOPMENT OF THE BLOOD VESSELS AND OF THE HEART IN FERRET EMBRYOS, WITH A COMPARISON BETWEEN THE CONDITIONS OBSERVED IN THE FERRET AND THOSE NOTED IN OTHER MAMMALS OF RELATIVELY THE SAME STAGE OF DEVELOPMENT.

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INTRODUCTION

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The object of the present investigations is to trace, as far as possible, the earliest stages of development of the main blood vessels and of the heart in ferret embryos. Before the details of the Reconstructions are given and the conclusions to which they tend are discussed, it will be profitable to consider briefly the investigations which have already been made regarding the formation of the intra-embryonic vessels and the early stages of the development of the heart in mammals generally. It may be pointed out at once that the phenomena observed are, of necessity, closely associated with the early stages of development of the pericardium and are connected with the mode of development of the pre-umbilical portion of the body of the embryo.

DEVELOPMENT OF THE BLOOD VESSELS.
(Review of Literature.)

In attempting to trace the first formation of the intra-embryonic blood vessels, it is necessary to begin with the development of the extra-embryonic blood vessels, indeed, to go back to the formation of the first rudiment of blood vessels and blood cells of the yolk-sac. The question of the origin

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of the vascular rudiment is one of the most obscure in the realm of comparative embryology. Even the lowest vertebrates, which present the greatest simplicity in their structures and whose development is most easily understood, have failed us in this question. For Hatschek(88) who has made exhaustive studies of the development of Amphioxus, has designated the blood vessels as the only system of organs concerning which he was unable to form any definite conclusion.

There has been great diversity of opinion regarding the germ layer from which the vascular endothelium and blood cells arise. In the literature it may be found that certain competent investigators have in each vertebrate class claimed the vascular endothelium and blood cells to be derived from entoderm, while other workers of equal authority have found the vessels and corpuscles to arise from the mesoderm. The consistency of the disagreements which are to be found in a review of this literature is most peculiar. These disagreements have their foundation mainly in the extreme difficulty of the problem when investigated on fixed or unfixed material. It is to be noted that in no case has an author stated that the blood cells and vascular endothelium are derived from different germ layers. Each author always takes the position that blood

cells and vascular endothelium arise from either the mesoderm or the entoderm.

Ziegler(87), in an earlier paper, expresses the view that "the system of blood vessels and that of the lymphatic vessels are produced in their first fundaments from remnants of the primary body cavity (blastocoel), which at the general distribution of the formative tissue(mesoderm) remain behind as vessels, lacunae, or interstices, and are closed by that tissue and incorporated in it". This author therefore agrees with Bütschli(82) that in all metazoa the blood vascular system has its origin from the blastocoel. On the other hand Felix(97), in his studies on the Salmonidae inclines to the belief that the circulatory system is, from a developmental point of view, closely related with the coelom. Ziegler further suggests that it may be that the blood rudiment in phylogeny has been passed to the mesoderm from the entoderm, and for this reason the entodermal origin may sometimes occur in coenogenetic development.

In the reptiles, Strahl(83), in the birds, Kölliker(84), in the selachians, Ziegler(92) and in mammals, Kölliker(84), all claim that the first vessel rudiments are found in the mesoderm and not between the mesoderm and entoderm. The current view which is held by most investigators is that in embryos of the higher vertebrates, the first

vascular/

vascular rudiments which can be identified as the fore-runners of the blood vessels and blood cells, appear, at first, in the form of localized cell cords lying upon the yolk-sac between the mesodermic tissue and the entoderm. His(00) gives the name of Angioblast to these localized cell cords. The genetical origin of the angioblast is sub judice.

Upon the question as to whether the angioblast is to be looked upon as a derivative of the mesoderm, or as an off-shoot from the entoderm, opinions again diverge. In support of the mesodermic origin, the names of Maximow(09), Weidenreich(10), Evans(12), Rückert and Mollier(06) are of prominence, while the advocates of the entodermic theory are to be found in the persons of Kölliker(82), Robinson(92), Keibel(88) and Van der Stricht(99). Miss Parker(15), in the course of her investigations into the development of the heart in marsupials, admits the possibility of the entodermal origin of the endothelium, for she finds clear evidence of proliferative activity on the part of the entoderm of the area vasculosa. Minot(12), however, inclines strongly to the opinion "that the mesoderm is formed first and that the angioblast, added later, not through transposition and transformation of mesodermic cells already present, but from cells which separate from the yolk, or from the layer of yolk

cells,

cells, and form a reticulate grouping of themselves between the middle and lower germ layers".

In all of the non-pelagic bony fish eggs investigated up to now, it is stated that the chief blood forming cells are, without exception, to be found in a definite mass of cells which, in the Teleost embryo, is very characteristic and is located in the caudal half of the body, between the notochord and the gut, extending well into the tail region. It is claimed that this so-called 'intermediate cell mass' forms both vessels and blood cells.

In Fundulus, Stockard(15) finds that vascular endothelium does arise in situ in many parts of the embryonic body in which blood cell rudiments are not present, and that independent blood islands, having no connection with the intermediate cell mass, are found on the yolk-sac, and even in extremely young embryos blood islands may appear on the ventral yolk surface at a great distance away from the intermediate cell mass. The yolk-sac vessels seem to arise by aggregation of wandering mesenchymal cells. Certain of these cells elongate and group themselves in such a way as to form vessel tubes. He states that after the vessels are formed they may then be seen to send off buds and sprouts to form new vessels, in the manner Clark(09) has described in Amphibians. The difference between the cells giving rise to the

vascular endothelium and those forming the blood cells is not distinguishable in early stages.

The persistent claims that vascular endothelium has the power to change into the various types of blood corpuscles have been disproved by the recent experimental work of Stockard(15). He finds in a series of experiments made on Fundulus embryos that the endothelium of vessels containing blood never presents any cell in a transitional stage. Stockard states that "if vascular endothelium had such a power, then one might expect that this power would show itself in cases where it was most needed, for example, in those embryos in which the blood has never circulated". (The circulation of the experimented embryos had been prevented from entering certain definitely formed vessels.) He states further with regard to the embryos experimented upon that "The heart and aorta and numerous vessels in the head and cranial portion of the body are lined with typical vascular endothelium, yet in no instance has it been found that one of these vessels contains a single red blood cell in any stage of development".

After considerable study and careful observation Stockard has observed nothing that would indicate that the vascular endothelial cells possess the power to change into the blood cell type, nor could he find any evidence to indicate that cells

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having once assumed even the earliest blood cell type are capable of metamorphosis to form endothelial cells. He firmly believes that, in Fundulus at least, endothelium is incapable of giving rise to any type of blood cell. Felix(97) also has noted the fact that the aortae in early normal Teleost embryos are invariably free of blood cells.

Whatever its origin, the angioblast, according to current views, is, in the majority of amniota, chick for example, found lying between the mesoderm and entoderm in the form of cell cords in the area vasculosa immediately surrounding the embryonic shield. It is believed that the peripheral part of each angioblastic strand soon resolves itself into an uninterrupted net-work of endothelium and the central part into clusters of blood cells. The area occupied by the angioblast is generally spoken of as the area vasculosa and can be recognized, in the vast majority of cases, by the red colour of the blood islands. The colorization of the area vasculosa marks the beginning of the development of haemoglobin. The endothelial cells which enclose the blood cells continue to divide and produce vascular sprouts which appear, at first, as solid cell cords but later become hollow (Hertwig(92), Minot(12)).

Simultaneously with the proliferation of the primary endothelium to form the vitelline plexus,

secondary vascular rudiments also appear independently upon the yolk-sac to augment the completion of the vitelline circulation, but a time is soon reached when the latter mode of development ceases, and subsequently all endothelium of the embryo is derived from pre-existing endothelium (Evans(12)).

Around the periphery of the area vasculosa, in the majority of animals, the vitelline plexus resolves itself into a broad circular vessel - the sinus terminalis, which is continuous round the margin of the area except at the cranial end where it terminates on each side in a vessel which enters the embryo. The vascularization of the splanchnic layer of the mesoderm gradually extends through the extra-embryonic region of the zygote until it covers the whole extra-embryonic region, where it forms an intermediate layer between the ectoderm and entoderm.

Some of the larger channels of the area vasculosa, even in the early stage of its formation, converge to form a single vessel on each side, which enters the embryonic body through the splanchnopleure and ultimately joins the venous ends of the heart rudiments when these are developed. These are the two vessels previously mentioned as connected with the cranial end of the sinus terminalis and are known as the omphalomesenteric(vitelline) veins.

It is believed that other channels of the area vasculosa on each side grow towards the median plane of the embryo and as they approach the region of the notochord their extremities fuse to form a longitudinal vessel which is the dorsal aorta of that side. The dorsal aorta ultimately unites with the arterial end of the heart. In this way the vitelline circulation of the embryo is completed and plays an important rôle in all vertebrates in supplying the growing embryo with nutritive materials from the yolk, which is comparatively large.

In reptiles and birds, a second circulation, as it were, develops in connection with the allantois, and persists during incubation, since the allantois is chiefly a reservoir for the waste products of the body. In mammals the allantois is rudimentary, its place being taken by the placenta which establishes the communication between the embryo and the mother, and the vessels which correspond to the allantoic vessels in reptiles and birds become associated with the placental circulation (vide infra).

The consideration has been confined so far mainly to the part played by vessels which in origin are extra-embryonic. The next question to be considered is whether the early blood vessels in the body of the embryo itself are formed by an ingrowth

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of the vitelline plexus which is, by origin, composed of extra-embryonic blood vessels, or, whether, on the other hand, the intra-embryonic vascular system, or at least a part of it, arises in situ from the germ layers of the body. The problem is difficult, and it is not surprising that various conflicting views have been formulated regarding the precise mode of origin of the intra-embryonic blood vessels. Thus, the names of His(00) and Hertwig(92) have been identified with the idea that the early blood vessels in the body of the embryo itself are formed by a budding or ingrowth of the endothelial wall of the vessels from the extra-embryonic vascular area, and the name of Sobotta(02) is associated with the belief that there is an outgrowth of vessels from the body of the embryo to the wall of the yolk-sac. Rabl(86), on the other hand, pointed out the possibility of the endothelium of the vessels of at least the head, if not the whole vascular system of the embryo, having been formed by the extension of the paired heart rudiments after the latter have made their appearance, whilst Rückert and Mollier(06) maintain that the embryonic vascular stems, or at least a part of them, arise in situ from the mesoderm of the embryo.

Sobotta(02) imagines a gradual growing away of

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the vascular system from the local origin, the sclerotom. The aorta is the primary vessel, and, for example, the sub-intestinal vein arises from the aorta by vascular sprouts which grow around the gut, broaden out and fuse on its ventral side and finally give rise to the longitudinal vein.

Other investigators basing their opinions on the results of a series of experiments on growing chick embryos, adhere to the conviction that, even after the destruction of the yolk-sac vessels of one side, the heart, the aorta and the other vessels are found to develop on both sides of the embryo. Stockard(15) favours the view that a definite mass of cells characteristic of the Teleost embryo, which is located in the caudal part of the body between the notochord and the gut, and which is called the 'intermediate cell mass' is the intra-embryonic vascular rudiment in many of the species.

Rückert(88) claims that in Selachians the aorta arises in loco. Felix(97) states that the glomerulus of the bird mesonephros originates in loco independently of the aorta, and believes that in the Salmonidae the stammvene, the venenplexus of the mesonephros, certain vessels of the glomerulus, and also the mesenteric artery along with the aorta, arise in loco. Regarding the rudiment of the heart and venae sub-intestinales, Felix is not certain,

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but thinks that these likewise arise in loco. All these observations are directly opposed to the theory of ingrowth of vessels from the yolk-sac, i.e. the parablast theory of His(75), as well as the outgrowth of vessels in the sense advocated by Sobotta(02).

In birds the intra-embryonic blood vessels have been investigated by Vialleton(92), His(00), and Evans(09), to whom we are indebted for a comprehensive knowledge of the formation of the caudal portion of the dorsal aorta. According to these authors, the fact appears to be established beyond doubt, that the greater part of the dorsal aorta in the bird is formed from the medial margin of the vitelline plexus which has grown into the embryo in the manner already referred to. Türstig(84) also has noticed the frequent early connection of the primitive dorsal aorta with the vitelline plexus in mammals. From this observation it seems highly probable that in mammals the caudal portion of the dorsal aorta has perhaps a similar origin.

With regard to the development of the cranial portion of the aorta, on the other hand, various opposing views are held; thus, His(00) attributes it to the result of a further growth of the same extra-embryonic vitelline plexus which forms the caudal part of the aorta, but which is reduced to

a capillary chain growing headwards, eventually turning ventrally over the blind end of the fore-gut and fusing with the cranial portion of the heart tubes; whilst Rückert and Mollier(06) maintain that the cranial portion of the aorta is developed in situ from the mesodermic cells of the lateral plate of the mesoderm of the cranial region of the embryo.

Lewis(04) observes that all intra-embryonic blood vessels of rabbits are apparently derived as off-shoots from the extra-embryonic net-work of vessels in the splanchnopleure of the yolk-sac, the vitelline plexus ending medially in the embryo in the form of two vessels - the dorsal aortae. Lewis therefore agrees with His as to the origin of these two vessels. Quite recently Bremer(12) arrives at a somewhat similar conclusion, namely, that in the rabbit embryo of 5 somites, the dorsal aorta, the first aortic arch, the conus arteriosus and the lateral heart are all parts of an original net-work of angioblastic cords derived from the extra-embryonic plexus of blood vessels.

Mollier(06), in his review regarding the origin of vessels, concludes as follows - "as to the genesis of embryonal vessels we may pass the judgment that the theory of the local origin of the vascular endothelium is valuable. The notion of His(75) and Vialleton(92) that the vessel strands of the embryo grow in as sprouts from the extra-

embryonal

embryonal rudiment(vascular rudiment) is not nearly so probable as that the individual vessel cells arise in loco and thus form the vascular nets".

This statement agrees in every respect with the contentions so fully presented by Huntington (10, 14), McClure(10, 12) and others, regarding the origin of lymph vessels. Recently it receives additional support from the experimental results recorded by Miller and McWhorter(14) on the origin of blood vessels in the chick embryo. Such a view is further strengthened by the still more recent experimental evidence presented by Reagan(15), which shows the origin in loco of vessels in isolated parts of chick embryos, and by Stockard(15), which claims that in Teleost embryos there can be no doubt that the heart endothelium and aortae arise in situ within the embryo, when in Teleost embryos there are no vessels, or even mesoderm, present on the yolk-sac in the cranial region of the embryo. Stockard(15), however, states that certain vessels do partially grow out from the embryo on to the yolk-sac, and other smaller vessels arise in many separate regions of the yolk-sac as the products of wandering mesenchyme cells which become arranged to form the tubular vessels, and that all of these vessels, after they have arisen, may grow by budding or by sprouting off new vessels, or may increase in

length/

length by a forward growth as described in living embryos by Clark(09, 12) in his careful observation on this subject.

It is to be noted that all these experiments just quoted confirm the earlier results of Hahn(09) on the origin of vessels in the chick, and that the formation of intra-embryonic blood vessels is much more extensive and important than has formerly been supposed.

DEVELOPMENT OF BLOOD CELLS.
(Review of Literature)

Though the development of blood cells lies without the scope of the present investigation, a few remarks may be made regarding the close relationship of these cells to the vascular endothelium. Nearly all investigators on this subject assert that blood cells and vascular endothelium arise from either the mesoderm or entoderm. In reviewing the literature on this point no statement has been found which might suggest that blood cells and endothelium develop from different germ layers. The possibility of these structures having an independent and separate origin cannot, however, be overlooked, as will be seen later.

Maximow(09) believes that the endothelial cells and blood cells are closely related, and arise from

a common stem cell in the blood island, and may continue to arise from such a cell during later development. Stockard(15), on the other hand, states "that vascular endothelium forms in perfect normal fashion within the heart region and head region of embryos without circulating blood, but in no case in early or late stages was the endothelial lining of the aorta or other vessels capable of giving rise to any type of corpuscles. Yet the power to form blood corpuscles was abundantly present in the same embryos as shown by the huge numbers of blood cells within the blood formimg regions - the intermediate cell mass and yolk islands".

Maximow(09) states further that the intravascular primitive blood cells are not only increased by mitosis but are added to also by the proliferation of the same kind of cells from the fixed endothelial wall of the primitive vessels. The assumption is based on the fact that clusters of blood cells are often seen adherent to the endothelial wall of blood vessels. Maximow thinks that these clusters of cells may arise by the proliferation of the endothelium. Minot(12), however, disagrees with Maximow because he finds that there is no continuity of the protoplasm of the cells either in the rabbit or in man; also, because mitosis of the endothelium in the neighbourhood of the clusters is almost invariably wanting;

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and, finally, because the endothelial nuclei are differentiated, while the nuclei of the cells of the clusters are not differentiated. Minot regards the cells composing the clusters as solely primary wandering cells. Stockard(15) asserts that endothelial lining is utterly incapable of giving rise to any form of blood cell.

DEVELOPMENT OF THE HUMAN VASCULAR SYSTEM. (Review of Literature)

In man, according to Evans(12), it is certain that long before any vascular rudiments are found in the body of the embryo, and at a period long before any somites are present, typical vascular rudiments are detected scattered irregularly and covering at first only the surface of the ventral pole of the yolk-sac; but, on account of the comparatively small size of the yolk-sac, the vascularization of the whole surface of the yolk-sac is soon completed. Minot(12), however, expresses the view that in man, on account of the small size of the yolk-sac, the vascular area covers the whole surface from the start. These vascular rudiments, as in other vertebrates already studied, make their appearance as nodular swellings of that part of the wall of the yolk-sac known as the area vasculosa,

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and are cell clumps lying between the mesoderm and entoderm. Very shortly after their appearance, the peripheral cells of these cell clumps, according to general belief, arrange themselves to form endothelium and the central ones to form blood cells.

As in mammals, before referred to, so in man, in connection with the vascularization of the yolk-sac, or, according to Fetzer(10), even at a period before any vascular rudiments on the yolk-sac proper can be distinguished, there develop, in the belly-stalk and chorion of the embryo, highly characteristic strands of spindle cells, which repeatedly exhibit the nature of having the appearance of a double row of nuclei and of possessing a distinct lumen. This has been observed by Graf Spee(96) in the embryo von Herff of .37mm. These cells, by virtue of their processes, seem to have a tendency to anastomose frequently among themselves and to constitute one tissue of a definite nature. The strands of spindle cells have been claimed to form endothelial cells eventually, on account of the fact that lumina are occasionally found in them, and that when these cells are traced and compared through successive stages of older specimens, difficulty would be encountered in distinguishing them from those which are gradually involved in the formation of blood vessels.

In young human embryos, without any vessels or blood islands on the yolk-sac, Jung(07) and Herzog (09) have called attention to the aggregation of cells, sometimes arranged round a lumen, situated at the periphery of the mesoderm of the yolk-sac and belly-stalk in the neighbourhood of the extra-embryonic area. In slightly older specimens with recognizable yolk-sac vessels, irregular spaces in the mesoderm, some lined with endothelium, some without any definite lining, have been observed by many authors, and recently Grosser(13) and Debeyre (12) have independently described, beside the irregular spaces, true blood islands in the belly-stalk near the allantois. In human embryo 1.17mm. described by Frassi(08) there is an abundance of well-formed vascular rudiments on the ventral surface of the yolk-sac, and Frassi states that with very little or no difficulty, vessels can be detected also in the belly-stalk and chorion.

The next phase of development of the human vascular system is illustrated by the well-known embryo Glaevecke 1.54mm. of Graf Spee(89, 96). Here again, as in the preceding stage, vascular rudiments are seen on the yolk-sac and in the chorion, but, in addition to these, it is possible to note the first intra-embryonic vascular rudiments. In the region of the heart these intra-embryonic vascular rudiments exhibit the characteristic appearance of endothelial

cells,/

cells, and in the more caudal portion of the embryo strands of clearly isolated and differentiated cells are found lying between the entoderm and the mesoderm; these probably represent intra-embryonic vascular cells, the mode of origin of which will be considered later. When these strands of cells are traced latero-caudally they seem to terminate on the upper surface of the yolk-sac. Finally, as the allantois is given off, they can be followed into vessels which in the belly-stalk lie at first on either side of and soon below the allantoic diverticulum. These vascular cells are, without doubt, to be looked upon as the rudiments of the umbilical arteries in the belly-stalk.

For the consideration of the next stage, the embryo studied by Eternod(95, 99) must be selected, as it exhibits the earliest known type of circulation in the human embryo; but a considerable gap exists between Spee's embryo and Eternod's, as will be seen later. Eternod's embryo measures 1.3mm. and possesses as yet no mesodermic somites nor intra-embryonic coelom. Eternod describes it as follows: "Le mésoderme forme au niveau du champ embryonnaire une lame compacte et indivise. Il se sépare seulement en dehors du champ embryonnaire en une lame externe ou amnio-choriale et une lame interne ou vitelline".

It exhibits paired heart rudiments cranially, which are simply dilated portions of the two sinus-like vessels - the venae umbilicales laterales. The two heart tubes are united for a short distance under the fore-gut into a single vessel which undoubtedly represents the future bulbus cordis and aorta ventralis. Two vessels sweep back from this one on each side of the notochord, which is still in the stage of the notochordal-plate; these are the primitive aortae, and the loops between the ventral bulb and the dorsal aorta are the first or primitive aortic arches, which, according to Eternod's description, are two or three in number on each side. (Evans doubts the interpretation of these arches and thinks that the picture given by Eternod is somewhat schematized, the small irregular vessels here likely being persisting strands of a capillary plexus.) Caudally, the aorta, sweeping past the neurenteric canal, bends round the caudal end of the embryo and runs into the belly-stalk to form the umbilical arteries, and out to the chorion as the chorionic capillaries. It may be mentioned that the aorta, throughout its course, is not represented as giving off any branches to the tissue of the body. The umbilical veins, which collect the blood from the chorionic plexus, unite in the belly-stalk into a

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single trunk(vena umbilicalis impar), but again separate as the embryo is reached and enter the embryo on either side near the attachment of the amnion. Where the belly-stalk becomes continuous with the yolk-sac, each umbilical vein is joined by a large tributary from the capillary plexus of the ventral surface of the yolk-sac, where these two tributaries anastomose with one another. In this way a venous ring is produced encircling the allantois. The significance of this ring is obscure at present. Though there are many vessels on the surface of the yolk-sac, especially on its ventral aspect, no indication of a vitelline circulation can yet be detected, for no communications between these vitelline capillaries and the aortae can be traced. The earliest stage of the development of the dorsal aortæ and the umbilical veins in man is still unknown, for the link between the Spee's embryo and the Eternod's is still wanting.

It appears, therefore, from the above mentioned evidence that there is a circulation set up between embryo and chorion through the umbilical vessels at a very early stage, before even the vitelline circulation is established and before the appearance of any mesodermic somites. This view seems to be further supported by the observations of Selenka(86), which show that in Hylobates rafflesi the vessels on the under surface of the yolk-sac communicate with the

vessels/

vessels of the chorion by a pair of vessels surrounding the allantoic tube, before there are any vessels in the embryo.

It is not difficult to understand why in man the umbilical or placental circulation should overshadow, in importance, the vitelline circulation, for it is to be recollected that the human yolk-sac is comparatively small in size and therefore contains an inadequate supply of nutritive material to meet the demands of the growing embryo. Hence, it is of the first importance that the circulation of the embryo once established should be connected with the chorion. This is another instance of the remarkable series of variations from the ordinary type which the development of the primate embryos exhibit.

In embryos of 5 somites and upwards it is observed that the vitelline plexus has established its communications cranially with the heart by means of two channels, the vitelline veins, reaching as far as the first intersegmental cleft, as Dandy(10) first showed, while caudally, in the region of the unsegmented mesoderm, the branches of the vitelline arteries form a plexus of capillary-like vessels from which, as shown by Felix(10) and confirmed by Evans(12), the umbilical artery takes its origin. This fact leads Evans to believe that the umbilical artery is to be looked upon as a modified vitelline vessel.

vessel. Cranially the vitelline plexus communicates on each side with the heart rudiments by two vitelline veins with which the umbilical veins have already established their connection.

From the conclusions arrived at by Bremer(14) it seems clear that in human embryos the earliest blood vessels appear separately in the yolk-sac and in the belly-stalk in the form of multiple rudiments. He believes that the rudiments in the belly-stalk and probably also in the yolk-sac in many cases are funnel-shaped invaginations of the surface mesoderm, but they may be solid cords of cells. By a partial fusion of the walls of a funnel-shaped ingrowth, a portion of the coelom bordered by mesoderm, may be cut off as a separate cavity - the cavity of a blood vessel, lying deep within the substance of the belly-stalk. According to this author new endothelium seems to arise either by delamination from the walls of such a detached portion of the coelom, or by direct extension of the wall, in the form of an angioblast cord from the mesothelial ingrowth. From the endothelium, by whichever method developed, further extension is by means of the angioblast cords, which grow apparently through the surrounding mesoderm.

DEVELOPMENT OF THE HEART AND PERICARDIUM.
(Review of Literature)

In mammals much has been done to throw light upon the development of the heart, notably by His (81, 85, 86) and Bischoff(42, 52), in rabbits, Born (89), in dogs, Bonnet(91, 01, 07), in pigs, Keibel (88), and in ferrets, Yeates(11), but in man very little is yet known, on account of the existence of many gaps which must be filled up before it is possible to obtain a clear conception of the human embryology, not only of the heart, but of many other important organs; and these, at the present day, can be carried out only by a series of investigations into the development of the various mammals suitable for the purpose.

Tandler(12) says "the earliest developmental processes of the heart, especially in so far as they concern the formation of the endothelium of the heart and vessels, are unknown in the human embryos, but probably one will not be far astray in assuming that the earliest rudiment of the human heart is essentially similar to that of the mammalia". The earliest stages of development of the mammalian heart is undoubtedly intimately associated with the development of the blood vessels, but concerning the latter various opposing views have been formulated

and already been dwelt upon in the beginning of this paper.

It is clear that the precise mode of development of the blood vessels is not yet definitely established, and a short survey of the early stages of the development of the heart will show that our knowledge of that subject also is deficient. In mammals, according to Mollier(06), the first rudiment of the heart is the appearance of a number of cells, which are discernable in embryos of 2 to 3 primitive somites. These vascular cells appear between the entoderm and mesoderm on both sides not far from the median plane of the embryo, at first in the distal portion of the head; and they are found much earlier in the amniota than the anamnia. They are responsible for the formation of the endothelium of the heart tubes only, the remaining constituents of the wall of the heart, that is the myocardium and the epicardium, being derived from that part of the visceral coelomic wall which has been designated by Mollier(06) the heart-plate or cardiogenic-plate.

It is generally held that the first aggregation of the vascular cells of the mammalian heart is paired and is situated ventral to the coelomic cavity. By a process not yet satisfactorily explained, spaces soon make their appearance in the vascular cell mass, and when these spaces coalesce, two endothelial heart

tubes/

tubes are thus formed, one on either side of the median plane of the embryo; this bilateral origin of the heart was observed first by Hensen(76). A fusion of the two endothelial tubes next takes place, and the unpaired heart tube is formed from the paired heart rudiments, but exactly how this fusion is brought about, opinions differ.

It was for long believed (Balfour(81), Hensen (76), Hertwig(92), Kölliker(61)) and it is still held by some (Tandler(12), Bryce(08), Bailey(12), Wilson(14), H. von W. Schulte(15) and others) that in mammals, as in birds, the two endothelial tubes, out of which the heart is formed, appear at a time when the lateral folds, which are said to form the ventral wall of the throat, are only just visible, that, as the lateral folds of the splanchnic walls increase, the two halves of the heart, enclosed within the hitherto symmetrical and laterally placed pleuro-pericardial cavities, become carried medially and ventrally until they fuse on the ventral side of the fore-gut, and that the heart is therefore provided, at least for a time, with a ventral and a dorsal mesocardium.

Balfour states that "the heart should only be formed as two tubes when it arises prior to the formation of the throat" (mammals), and, "as a single tube when formed after the formation of the throat"

(elasmobranchii,

(elasmobranchii, amphibians). He concludes by saying that "the formation of the heart as two cavities is a secondary mode of development, which has been brought about by variations in the period of the closing in of the wall of the throat".

In the chick, on the other hand, a ventral mesocardium is recognizable, but this is due, as Robinson(02) points out, to the relatively late penetration of the mesoderm in the cranial region. In amphibians lateral folds have been described, but it is erroneous to presume that such folds, which, by virtue of their fusion ventrally, form the ventral wall of the fore-gut, really occur in mammals. In the latter, the pericardial mesoderm appears in the pericardial portion of the embryonic area, and it is there completely differentiated into somatic and splanchnic layers before the head bend is developed; there is therefore a single pericardial cavity to begin with, which extends from side to side along the cranial boundary of the embryonic area. As the head bend develops, the single pericardial cavity is reversed, and it is carried into the ventral wall of the fore-gut, where it forms a U-shaped tube which communicates at each end with the general coelom. The heart rudiments are formed in the splanchnic layer of the pericardial mesoderm; therefore, after the reversal of the area they lie in the dorsal wall of the pericardial cavity attached only by a dorsal

mesocardium to the ventral wall of the fore-gut, but they are never, at any time, connected with the ventral wall of the pericardium by a ventral mesocardium. This view is held also by Völker

Rouvière(04), on the other hand, while he agrees with Robinson as to the absence of a ventral mesocardium in mammals, gives a different account of the process which leads to the closure of the fore-gut. He describes the formation of the lateral pleuro-pericardial canals, which grow cranially round the cranial end of the brain-plate and fuse to form a continuous channel. The splanchno-pleure forming the caudal wall of the pleuro-pericardial cavity now forms a continuous fold, which Rouvière calls the "cardial fold" and which he describes as growing actively backwards as a whole. Gräper(12), in a description of the growth processes in the developing chick, which he worked out by staining the living embryos and keeping them under observation while still alive, shows that there is considerable evidence in support of the view, that the margin of the fore-gut(umbilical orifice) moves caudally concurrently with the growth of the head fold cranially.

Miss Parker(15), in her studies of the early stages in the development of Marsupials, summarises her statement by saying; "that while the initiation

of

head-fold formation is in all probability due to the forward growth of the brain-plate, there occurs also an active backward growth of the anterior intestinal portal(umbilical orifice). This process is associated with the rapid expansion of the pericardium which occurs at this period of development, and which brings about the backward and inward growth of the layer of the splanchno-pleure limiting the pericardium". She further claims that "the approximation of the heart tubes after gut-closure is due to the fact, that at this period the pericardium grows rapidly in length and decreases in width so that the heart tubes are brought together by longitudinal stretching of the pericardial wall lying between them".

The growth of the pericardium may exert, under certain conditions, a certain amount of mechanical influence on the shape and position of the heart tubes, for Stockard(15) finds in experimental Fundulus embryos that in those embryos in which the pericardium is hugely distended with fluid, the head end of the embryo is abnormally pushed far away from the surface of the yolk. The heart is thus stretched into a long straight tube or string leading from the ventral surface of the head through the great pericardial cavity to the anterior yolk surface. It is

to

to be noted that the above condition appears to be pathological. Normally the rate of growth of the heart tubes invariably exceeds that of the pericardium, so that the heart tubes are consequently thrown into loops. Any shifting of the two fixed ends of the heart tubes due to an undue rapid growth of the pericardium either cranially or caudally would, at first, undo the loops of the heart tubes or prevent them from forming, before exerting such an influence as to bring the two tubes into contact. There is no evidence to show that the pericardium grows more rapidly than the heart tubes.

Shore(89) describes that the head-fold of the chick embryo results from a growth of the head forwards over the diblastic part of the blastoderm, and that a 'folding off' does not occur, at any rate at first.

Recently Watt(15), in his investigation into two young twin human embryos with 17 - 19 paired somites, states: "From the atrial canal the ventricle continues on at the left and runs far forward in the pericardial cavity, when it is strongly flexed ventrally and turns caudad as it reaches the median line. It is here attached to the pericardial wall by a short stretch of ventral mesocardium, the only portion of this structure which is still present".

Though

Though a small piece of dorsal mesocardium is depicted in his paper(plate 3. fig. 4, and plate 4. fig.2) there is nothing to indicate the existence of the ventral mesocardium which Watt describes.

The following ferret embryos, which form the basis of the present communication, belong to the extensive collection of ferret embryos of Professor Arthur Robinson, Edinburgh University, under whose personal supervision I undertook to investigate, at the Research Laboratory, Anatomy Department, the early stages of development of the heart, and the main blood vessels and blood cells in ferret embryos in the Spring of 1912, with the object of elucidating, if possible, the points upon which there is difference of opinion with regard to those stages of development in mammals.

To facilitate future references to be made hereafter in this communication the ferret embryos are classified into stages; thus, in Stage I the description deals principally with the blood cells and vascular endothelium, in Stage II the account relates chiefly to the first appearance of the heart rudiment as a single transverse vascular channel situated caudo-ventral to the pleuro-pericardial cavity, in Stage III it is shown how the single heart tube is converted into two lateral heart tubes, in Stage IV a devotion is given mainly to the description of the conditions of the two endothelial tubes and their relationships to the muscular wall of the heart, and in Stage V the site of fusion of

the

the two heart rudiments to form an unpaired heart tube is indicated. At the end of Stage V a short description of a very early human ovum is given.

As far as the technique and the histological conditions of the ferret specimens, to be immediately described, are concerned, the description given in Stage IV may be applied equally well to all the other specimens selected for the purpose of this investigation. The only differences to be found in this respect are chiefly in the matter of staining and in a few other minor points, such as, whether the embryo is detached or not from the uterus before being sectioned.

STAGE I.

***

The material for this stage consists of 3 embryos. Selected sections of each of these specimens have been photographed to show the conditions and relationships of the blood cells and vascular endothelium.

(a). FERRET EMBRYO 1.15mm. (F.C.Q.Z.(Z)).

General Description.

The germinal area of this embryo measures 1.4mm. No mesodermic somites can be detected and there is no indication of a heart rudiment. The head-fold has not yet begun to develop and no intra-embryonic blood vessels can be found in the specimen.

There is, however, a shallow neural groove which terminates at the primitive caudal and subsequently dorsal end of the bucco-pharyngeal membrane. The groove broadens out caudally. Beneath the caudal end of the chordal plate, it becomes the chorda canal. The chorda canal terminates caudally in a mass of cells which fuse with the ectoderm at the cranial end of the primitive streak.

Blood Cells.

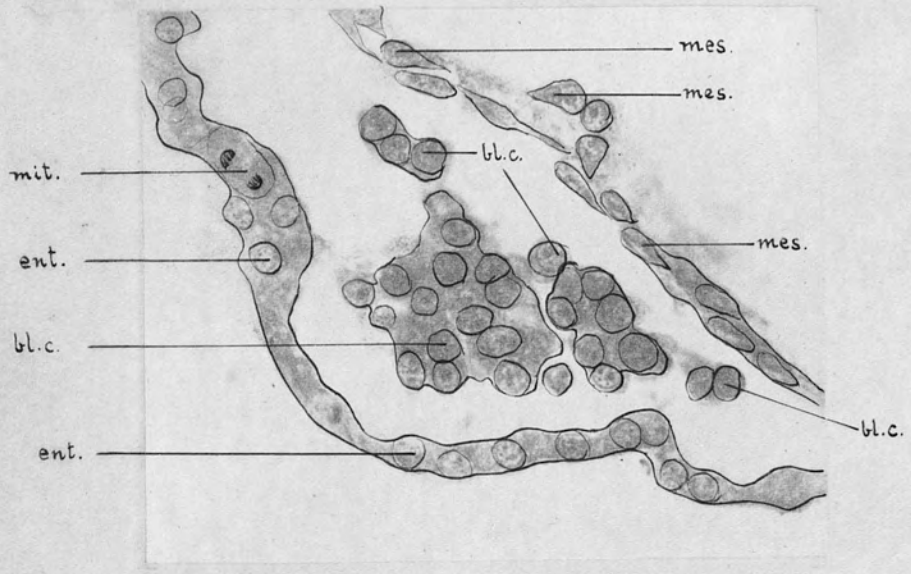
Though no intra-embryonic blood vessels can yet be found in this specimen, extra-embryonically there are solid clusters of blood cells which are not surrounded by any endothelium (fig.1). In none of the clusters of blood cells is it possible to detect that the peripheral layer of the cells resolves into endothelium. The cells forming the clusters are spheroidal in shape lying between the mesoderm and the entoderm. They are provided with large well-stained nuclei and are very often adherent to the entodermal cells which exhibit characters similar to those of the blood cells. No lumen can be found in any of the blood clusters.

The mesodermal cells spread out in a thin layer to cover the adjacent yolk-sac. They are spindle shaped and are attached to each other by long protoplasmic processes.

Figure 1. shows a portion of the extra-embryonic area in which a cluster of blood cells is seen lying free between the mesoderm and the entoderm. The cells forming the mesoderm are, as noted, spindle shaped, whilst the cells constituting the entoderm are spheroidal and exhibit other characters which are similar to those of the blood cells.

Mitotic

FIGURE 1. X 500.



bl. c. - Blood cells.

mes. - Mesoderm.

mit. - Mitosis.

ent. - Entoderm.

Mitotic division occurring in one of the entodermal cells can be detected in the neighbourhood of the blood cluster (fig.1). It is proved that this is not a singular occurrence by the fact, that a similar phenomenon is again seen in the entoderm of another embryo (Stage IIb. figs. 15a. and 17a.& b.).

(b). FERRET EMBRYO 1.6mm. (F.1904. QZ. U2).

General Description.

This embryo measures 1.6mm. after it has been cut. There is as yet no mesodermic somites. The heart rudiment is absent; and no intra-embryonic blood vessels can be detected. There is, of course, no head-fold.

A primitive streak is, however, present, and there is a primitive groove.

The notochord is tubular at its caudal end. In parts its ventral wall opens into the yolk-sac. Its caudal extremity is fused with the ectoderm at the cranial end of the primitive streak as in the previous specimen. Cranial to the primitive streak a faint neural groove is present on the surface of the embryo. It terminates, as in the previous case, at the bucco-pharyngeal membrane cranially.

Blood cells.

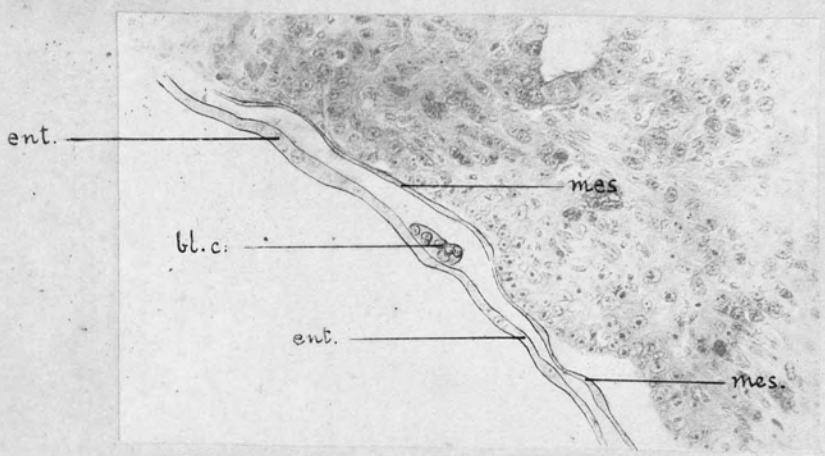
In this specimen blood cells are found abundantly on the wall of the yolk-sac between the mesoderm and the entoderm. These cells are spheroidal

in

shape provided with large well-stained nuclei as in the preceding specimen. They are arranged in solid clusters without any lumina in them and are devoid of any endothelial coverings(figs. 2a. & b. and 3). The majority of the cell clusters are found to be adherent to the entodermal cells which exhibit characters similar to those of the blood cells (fig. 2b.).

The mesodermal cells, covering the adjacent yolk-sac and in the neighbourhood of the blood cells are arranged in a thin layer which does not come into contact with the entoderm. They are spindle shaped and are connected with each other by long protoplasmic processes as previously noted (fig. 2a).

FIGURE 2a. X 200.

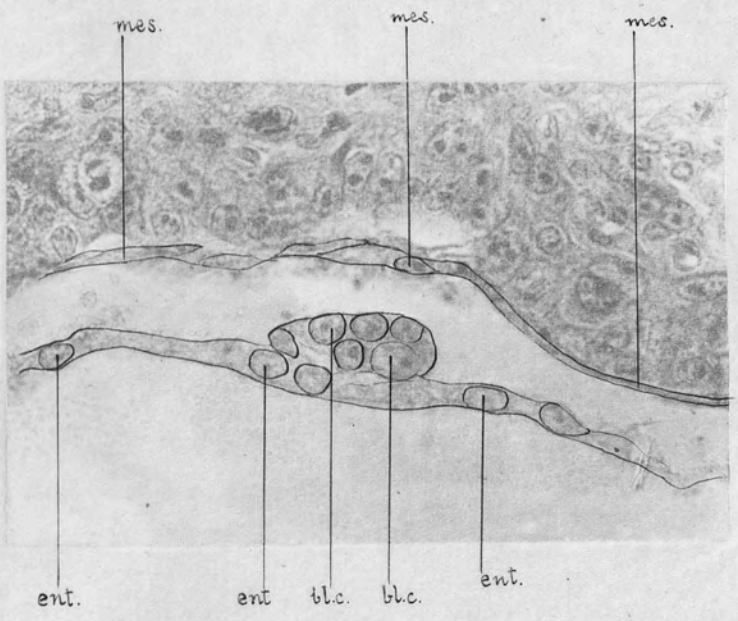


bl. c. - Blood cells.

mes. - Mesoderm.

ent. - Entoderm.

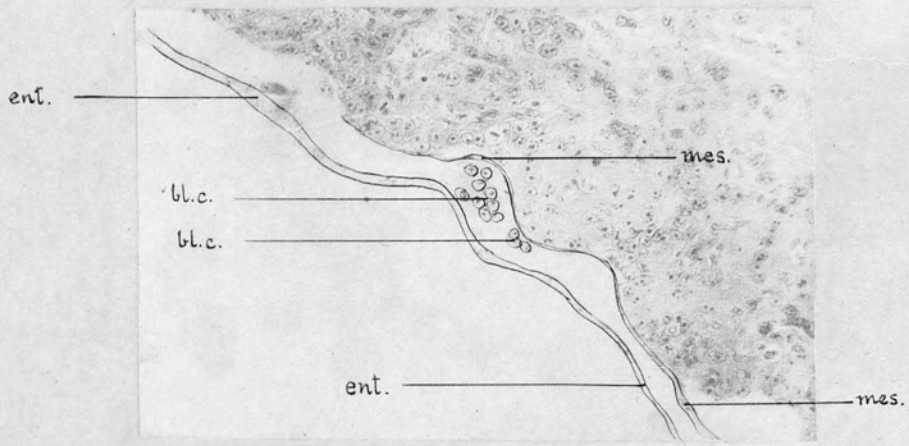
FIGURE 2b. X 500.



Same as figure 2a.

(Blood cells adherent to the entoderm)

FIGURE 3. X 200.



bl. c. - Blood cells.

mes. - Mesoderm.

ent. - Entoderm.

(c) FERRET EMBRYO 1.74mm. (F.1904. Q.A.A.U1). with 3 Somites.

General Description.

The primitive streak is well marked and is notched at the caudal part of its extent by the primitive groove.

The mesoderm, covering the caudal part of the embryonic area, is thickened and indicates the position of the allantoic mesoderm. Cranially the mesoderm of the primitive streak fuses with the caudal end of the chorda.

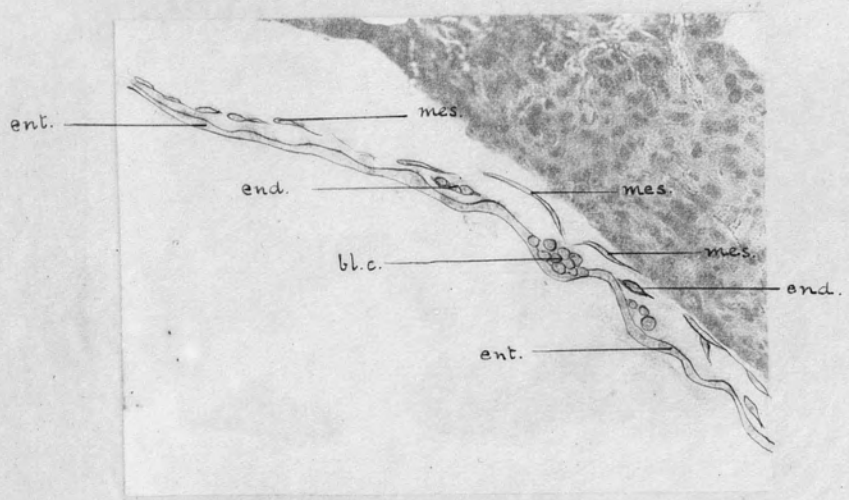
There is a broad shallow neural groove which narrows cranially and its walls become much thickened in the position which is occupied by the trigeminal ganglion. The groove gradually disappears and becomes continuous cranially with the buccopharyngeal membrane.

There is no heart rudiment. The two pleuropericardial canals are present one on each side of the embryo, but these have not yet grown across the median plane cranially; consequently there is no pleuro-pericardial cavity. No intra-embryonic blood vessels can be detected.

Blood cells and Endothelium.

Extra-embryonically clusters of blood cells are found scattered over the greater part of the yolk-sac to which they are often adherent (figs. 4a. & b.). The characters of the blood cells, the entoderm and the mesoderm are similar to those already seen in Stage Ia and b. specimens. The mesodermal cells (fig.5) are, however, more flattened and are connected with each other with longer protoplasmic processes than those observed in the previous specimens. In addition to the blood cells, endothelial cells can be detected here and there in the extra-embryonic region lying between the mesoderm and entoderm. These endothelial cells, unlike the blood cells, are spindle shaped (fig. 4) and can be traced in some cases to their mesodermal origin.

FIGURE 4a. X 200.



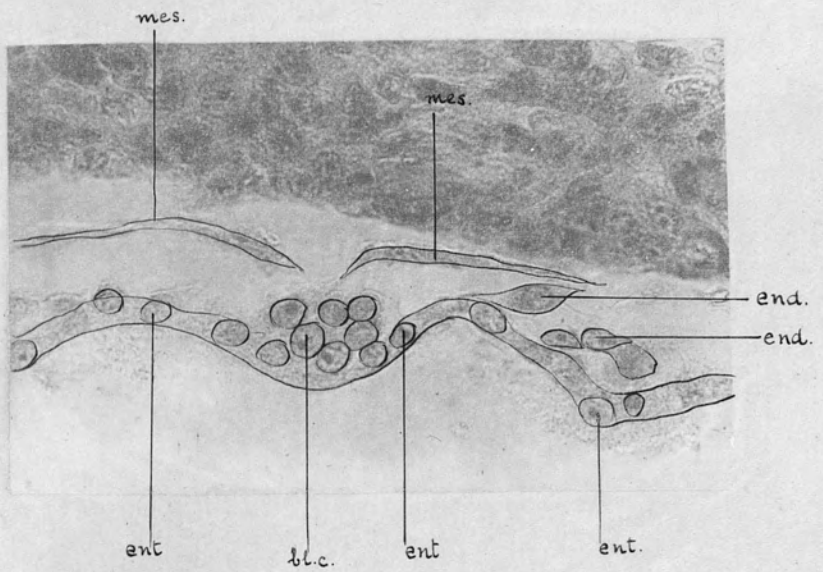
bl. c. - Blood cells.

mes. - Mesoderm.

ent. - Entoderm.

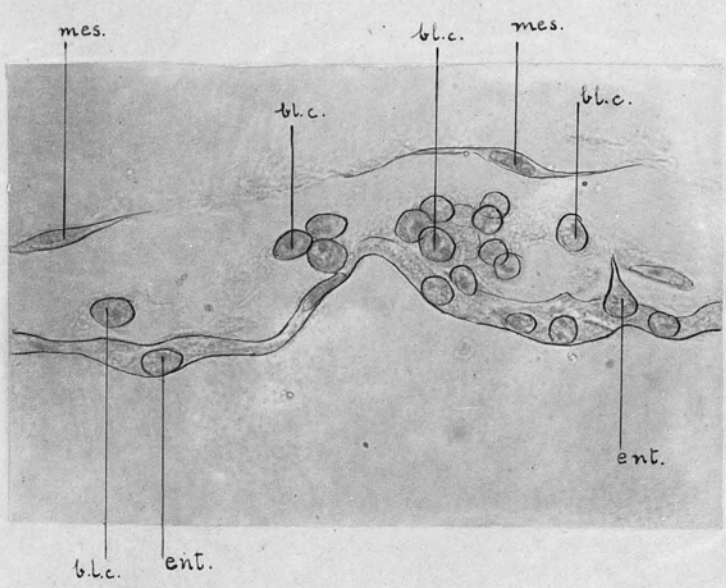
endo. - Endothelium.

FIGURE 4b. X 500.



Same as 4a.

FIGURE 5. X 500.



bl. c. - Blood cells.

mes. - Mesoderm.

ent. - Entoderm.

STAGE II.

***.

The material for this stage consists of 2 embryos one measuring 1.97mm. in length with 5 somites and the other, 2.3mm. with 6 somites.

(a)

DESCRIPTION OF THE GRAPHIC RECONSTRUCTION OF THE HEART AND CRANIAL PORTION OF A FERRET EMBRYO 1.97mm. IN LENGTH WITH 5 SOMITES. (F.B.A.A.).

This is the youngest specimen of the series of ferret embryos selected for the purpose of reconstruction in this investigation. Its total length measures 1.97mm. after preparation and embedding. It may be mentioned that the sections, each of which is 10μ in thickness, are perfect, and that the histological condition is excellent.

General Description.

No plastic reconstruction of the embryo was made, for it appeared that a graphic reconstruction of the heart would be sufficient for the purpose in hand

hand.

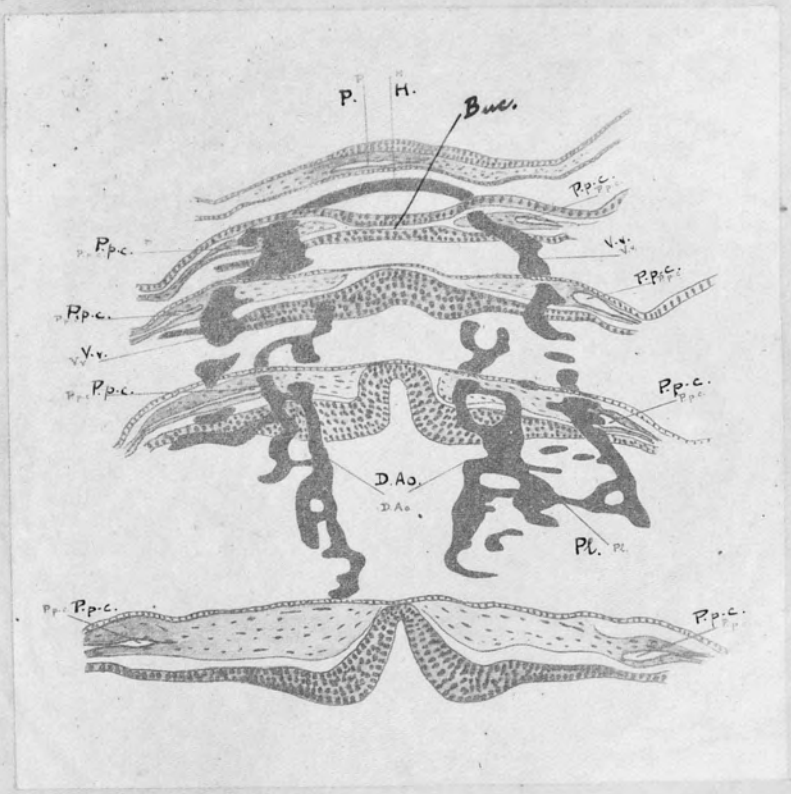
The neural groove and the primitive streak are both present. The neural tube is deepest at the brain region where it shows thickenings which correspond to the positions of the trigeminal and otic ganglia. The cranial end of the neural groove gradually shallows until it disappears at the primitive caudal end of the bucco-pharyngeal membrane.

Vascular System.

In this specimen the heart is represented merely by a transverse blood channel which lies across the median plane and unites the cranial ends of the two vitelline veins (figs. 6a.b.& c.). It is bounded caudally* by the bucco-pharyngeal membrane, and cranially by the pleuro-pericardial cavity (fig. 6a). The cranio-caudal diameter of the heart rudiment is 20 μ., its breadth, 120 μ.. The rudiment of the pleuro-pericardial cavity is present. It is that portion of the coelomic space which crosses the median plane of the embryonic area cranial to the rudiment of the heart and is closed cranially and caudally, and on each side it is connected with a pleuro-pericardial canal (figs. 6a. 7a.& b.). Together with the pleuro-pericardial canals, it forms

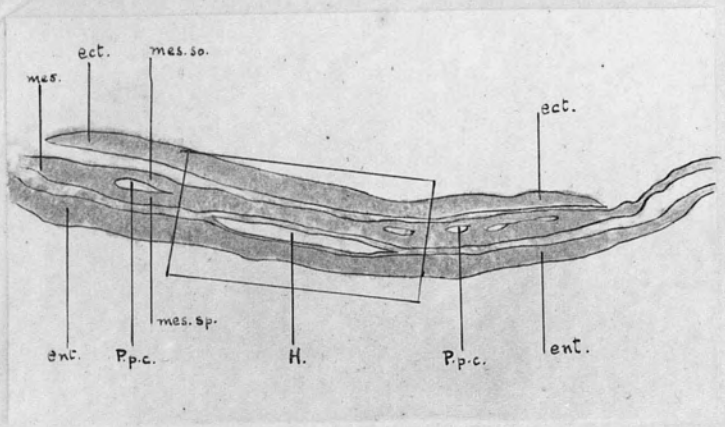
* The terms cranial and caudal are synonymous with anterior and posterior.

FIGURE 6a. X 500.



P. - Pericardium.
H. - Heart rudiment(primary union).
P.p.c.- Pleuro-pericardial canal.
V.v. - Vitelline vein.
D. Ao.- Dorsal aorta.
Pl. - Plexus.
Buc. - Bucco-pharyngeal membrane.

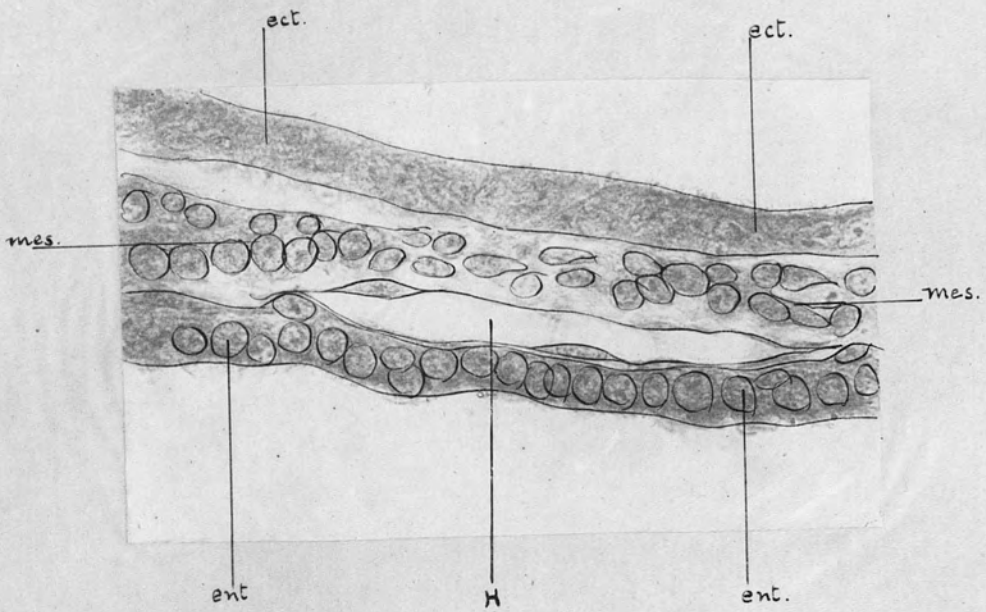
FIGURE 6b. X 200.



H. - Heart rudiment(primary union).
P.p.c. - Pleuro-pericardial canal.
ect. - Ectoderm.
ent. - Entoderm.
mes. - Mesoderm.
mes.so.- Somatic mesoderm.
mes.sp.- Splanchnic mesoderm.

FIGURE 6c. X 500.

(Magnified from the Square in Fig.6b.).



Same as in Fig. 6b.

forms an inverted U-shaped canal (fig. 6a.) which lies dorso-cranial to the vitelline veins and the heart rudiment (fig. 8.).

Each vitelline vein is placed ventral to the corresponding pleuro-pericardial canal and lateral to the dorsal aorta of the same side (figs. 6a. and 9.). The two veins converge cranially, and each terminates in the corresponding end of the heart rudiment cranial to the bucco-pharyngeal membrane (fig. 6a.).

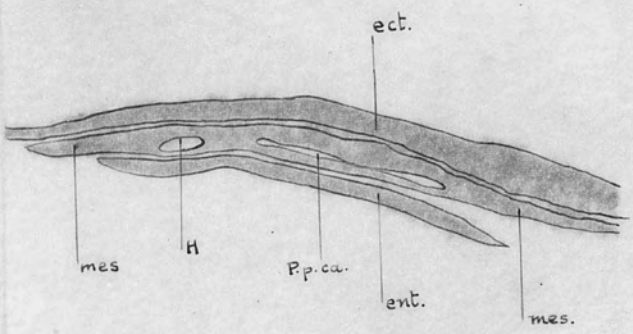
There are two rudiments of the dorsal aortae (fig. 6a.). They run caudo-cranially one on each side of the medullary groove. They are still more or less plexiform in character, and they terminate blindly at their cranial extremities some distance caudal to the heart rudiment.

Communications between the dorsal aortae and the corresponding vitelline veins are described by Bremer(12) in a 3.4mm. rabbit embryo. Such communications (fig. 6a.) can be traced in the caudal portion of the ferret embryo at this stage, which, so far as its general development is concerned, is considerably younger than Bremer's rabbit embryo. Caudally the dorsal aorta breaks up into a plexus spreading over the wall of the yolk-sac.

There is, of course, no ventral mesocardium,

and

FIGURE 7a. X 200.



ect. - Ectoderm.

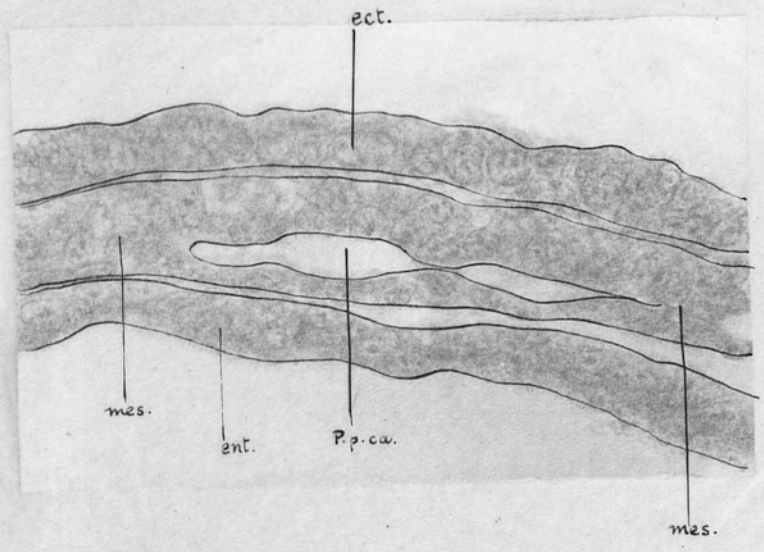
ent. - Entoderm.

mes. - Mesoderm.

P.p.ca. - Pleuro-pericardial cavity.

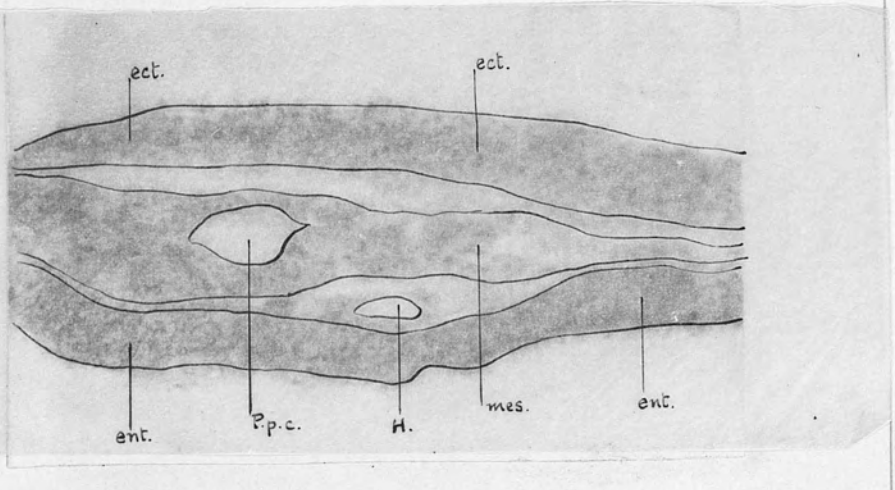
H. - Heart Rudiment.

FIGURE 7b. X 500.



Same as Fig. 7a.

FIGURE 8 X 500.

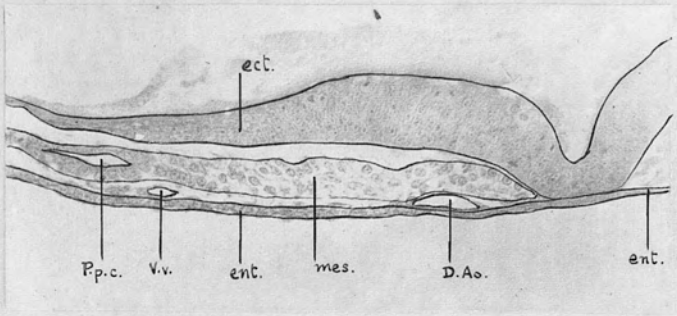


ect. - Ectoderm.
ent. - Entoderm.
mes. - Mesoderm.
P.p.c. - Pleuro-pericardial canal.
H. - Heart rudiment.

and the head-fold and fore-gut are not yet developed.

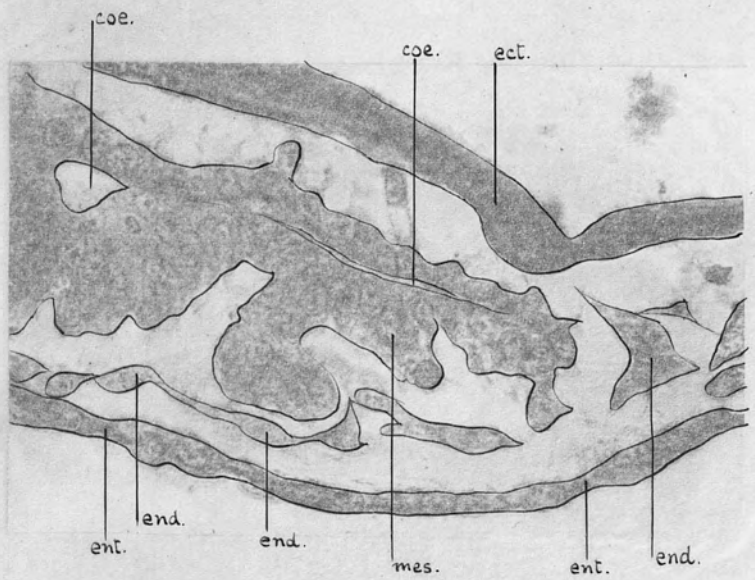
As far as the pericardial cavity and the pleuro-pericardial canals are concerned, this specimen does not differ, to any great extent from what has been described in Dasyurus viverrinus (7.5mm. vesicle) by Miss Parker(15). The heart of the ferret embryo is, however, in a more advanced stage of development than the 7.5mm. Dasyurus viverrinus, in which the heart rudiment is represented merely by some scattered angioblast cells and strands of cells, and in which the vitelline veins terminate cranially at the level of the caudal limit of the trigeminal rudiment. In this ferret specimen there is evidence to show that, in many of the sections, the vitelline plexus can be traced to its origin from the splanchnic mesoderm (figs. 10a. b. c. and d.).

FIGURE 9 X 200.



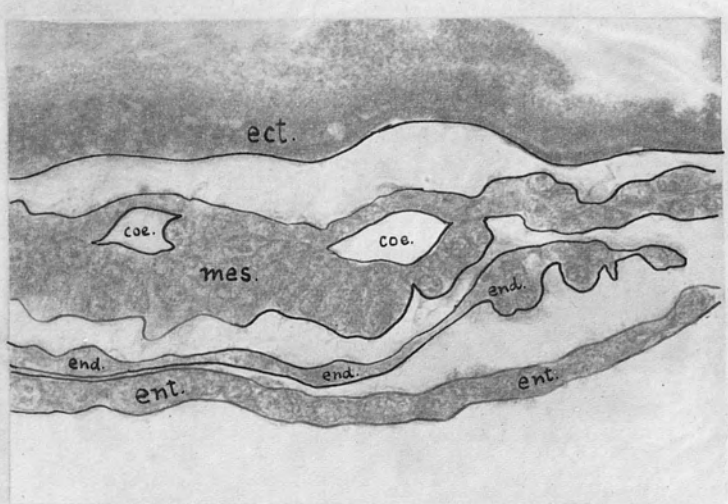
ect. - Ectoderm.
ent. - Entoderm.
D.Ao. - Dorsal aorta.
P.p.c. - Pleuro-pericardial cavity.
V.v. - Vitelline vein.
mes. - Mesoderm.

FIGURE 10a X 500.



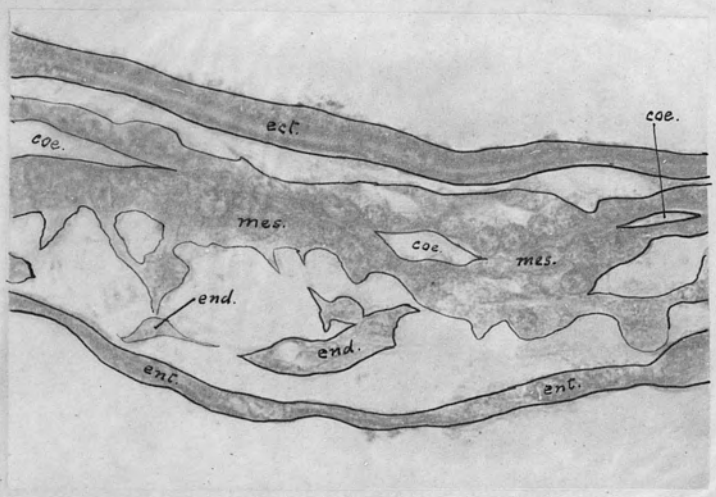
ect. - Ectoderm.
ent. - Entoderm.
mes. - Mesoderm.
V.pl. - Vitelline plexus.
coe. - Coelom.
end. - Endothelium.

FIGURE 10b. X 500.



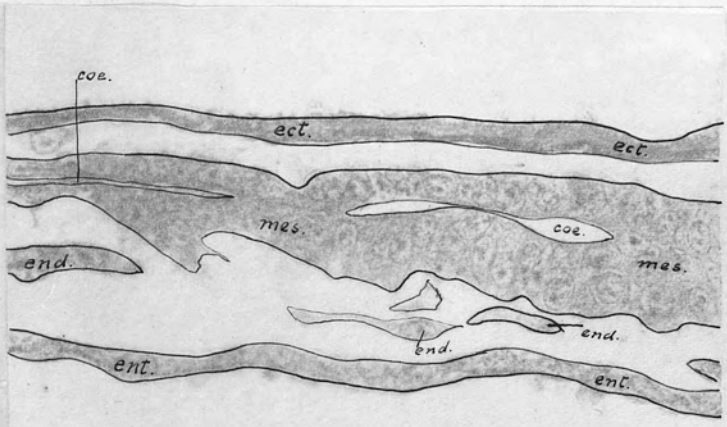
Same as in Fig. 10a.

FIGURE 10c. X 500.



Same as in Fig. 10a.

FIGURE 10d. X 500.



Same as in Fig. 10a.

(b).

DESCRIPTION OF A FERRET EMBRYO 2.3mm. WITH 6 PAIRS OF SOMITES. (F.1904. B.).

General Description.

The primitive streak is present. Its cranial extremity is continuous with the notochord which shows indications of the notochordal canal, and the notochord has begun to dovetail with the entoderm. More cranially the notochord is entirely fused with the entoderm, and for some 10 sections cranial to the primitive streak it can scarcely be distinguished except by the height of the cells from the entodermal cells.

The neural groove extends caudally to the primitive streak. It is wide and shallow at the caudal end, but deepens and narrows as it passes cranioward between the laterally placed mesodermal somites, and cranial to the somatic region the walls of the neural groove shows thickenings which correspond in positions to the auditory and trigeminal areas. The neural groove then gradually shallows until it disappears at the caudal end of the buccopharyngeal membrane.

Vascular System.

This embryo presents several features which have been observed in the preceding specimen. Though no reconstruction was made at the time, it was found, however, that with a study of the serial sections, many of the important structures can, with ease, be made out. As regards general development, this specimen shows only a slight advance on the previous one. The heart and pericardium (fig. 11a.) have practically not changed, the former lying ventral to the latter. Figures 11a.& b. illustrate the union of the two heart tubes across the median plane, and immediately cranial to this, the pericardium is seen stretching transversely to communicate on either side with the pleuro-pericardial canal (figs.12a.b.& c.). In the 1.97mm. ferret embryo (Stage IIa.), it has been noted that there is a communication between the vitelline vein and the dorsal aorta. Such a communication is seen also in this specimen (fig. 13.). Some of the intra-embryonic vascular endothelium can be traced to their origin from the mesoderm (figs. 14a. & b.).

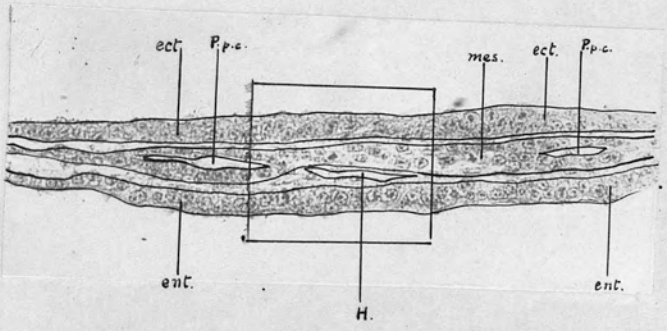
In this specimen it is possible to detect that some of the intra-embryonic blood cells are devoid of endothelium (figs. 15a. & b.), whilst others are partially engulfed by flattened vascular endothelium (fig. 16.).

Figure

Figures 15b., 17a. & b., taken from sections of the caudal end of this specimen show distinctly mitotic divisions in the entodermal cells in the neighbourhood of some blood cells.

There is no ventral mesocardium, and the fore-gut has not begun to develop.

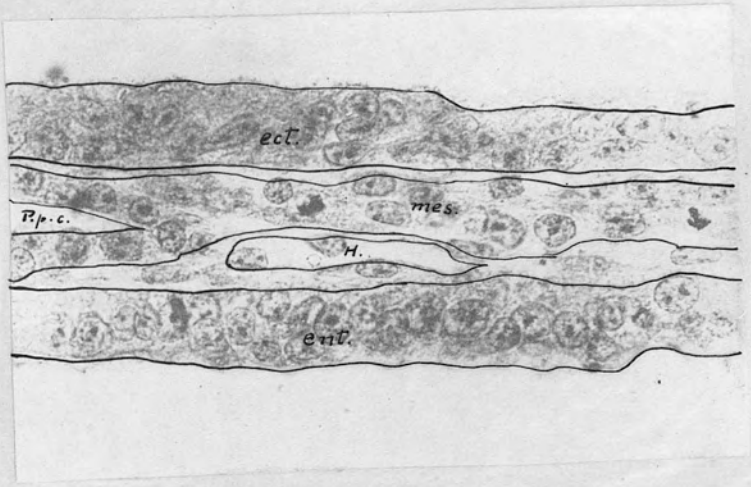
FIGURE 11a. X 200.



ect. - Ectoderm.
ent. - Entoderm.
mes. - Mesoderm.
P.p.c. - Pleuro-pericardial canal.
H. - Heart rudiment.

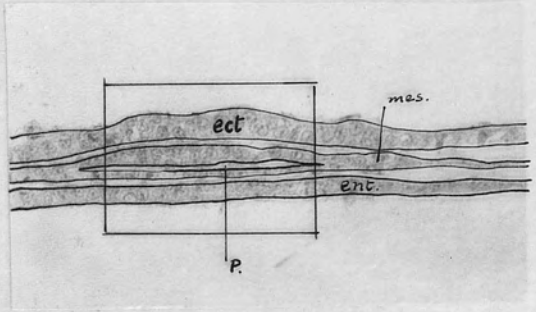
FIGURE 11b. X 500.

(Magnified from the Square in Fig.11a)



Same as in Fig. 11a.

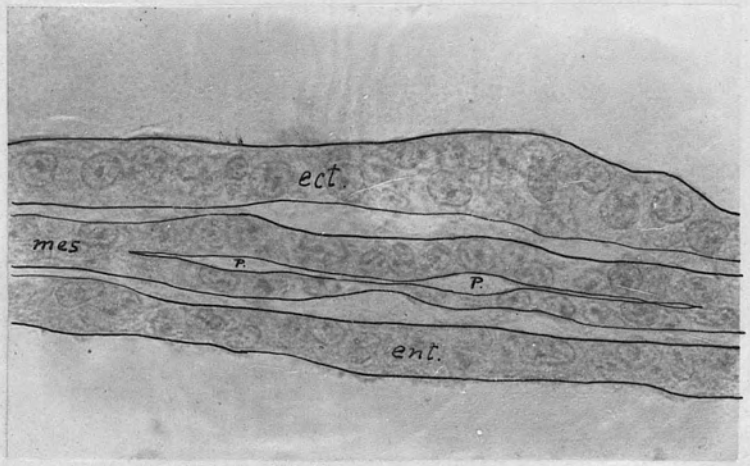
FIGURE 12a. X 200.



ect. - Ectoderm.
ent. - Entoderm.
mes. - Mesoderm.
P. - Pericardium.

FIGURE 12b. X 500.

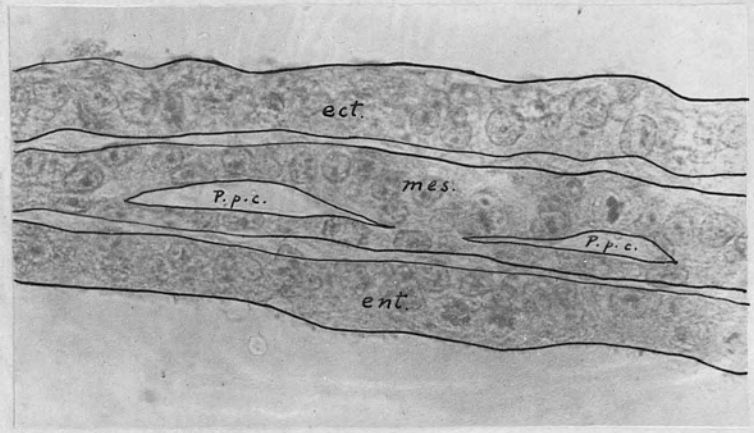
(Magnified from the Square in Fig. 12a)



Same as in Fig. 12a.

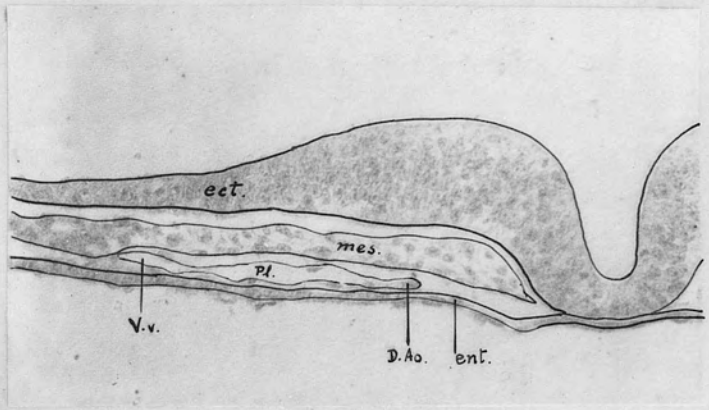
FIGURE 12c. X 500.

PLEURO-PERICARDIAL CANALS PLACED CLOSELY TOGETHER BUT NOT FUSED ACROSS.



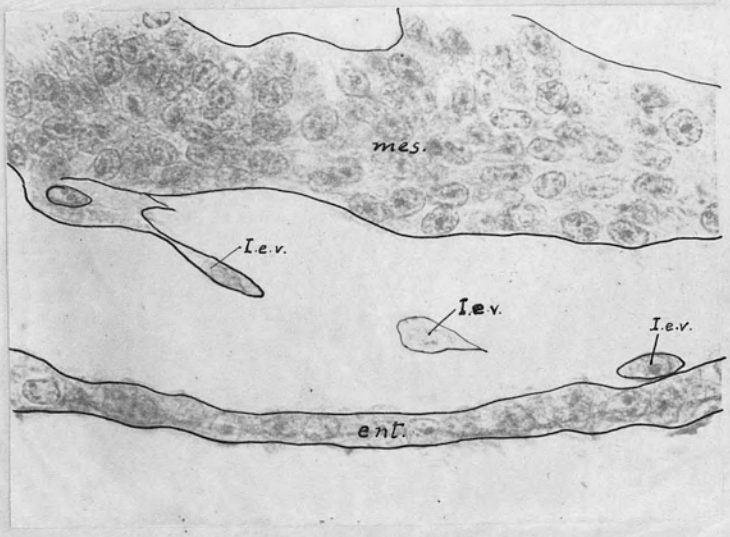
ect. - Ectoderm.
ent. - Entoderm.
mes. - Mesoderm.
P.p.c. - Pleuro-pericardial canal.

FIGURE 13. X 200.



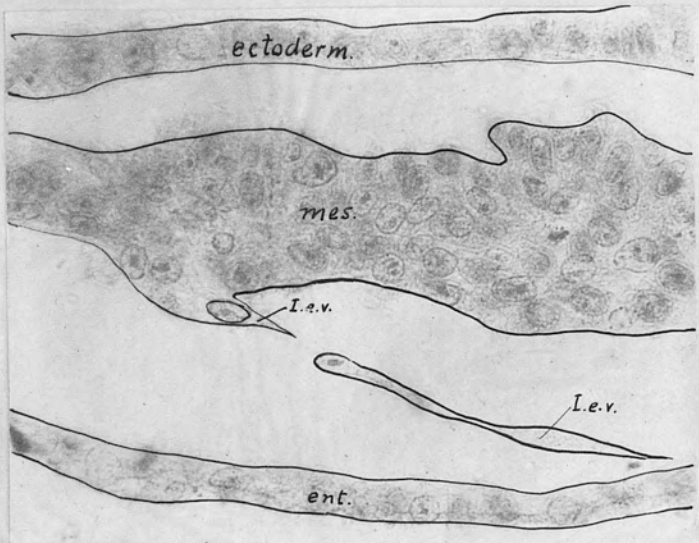
ect. - Ectoderm.
ent. - Entoderm.
mes. - Mesoderm.
D.Ao. - Dorsal aorta.
V.v. - Vitelline vein.
Pl. - Plexus.

FIGURE 14a X 500.



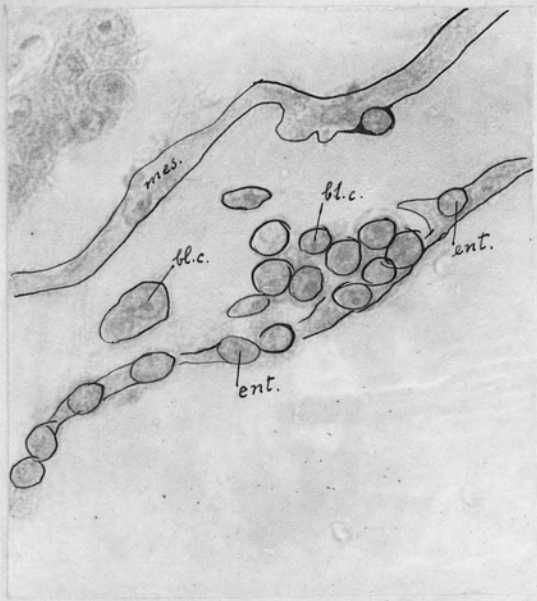
ent. - Entoderm.
mes. - Mesoderm.
I.e.v. - Intra-embryonic vascular endothelium.

FIGURE 14b. X 500.



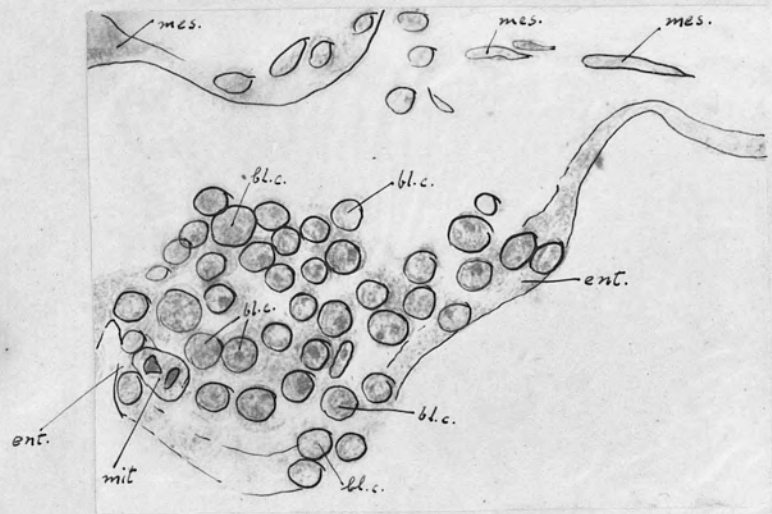
Same as in Fig. 14a.

FIGURE 15a. X 500.



ent. - Entoderm.
mes. - Mesoderm.
bl.c. - Blood cells.

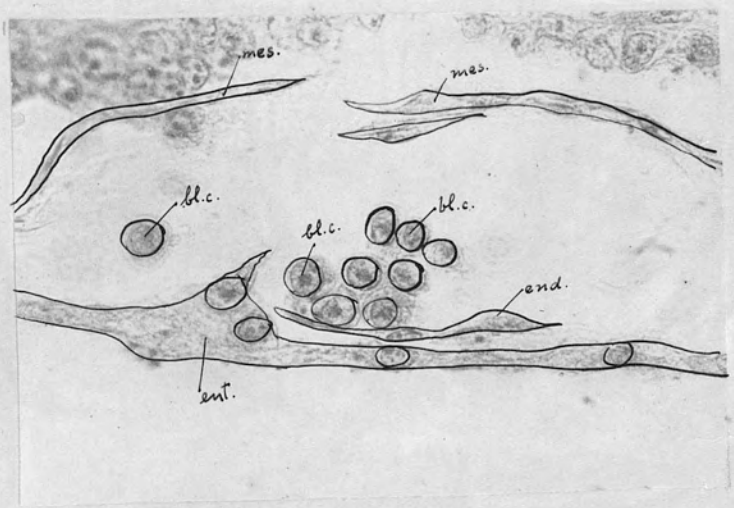
FIGURE 15b. X 500.



ent. - Entoderm.
mes. - Mesoderm.
bl.c. - Blood cells without endothelium.
mit. - Mitosis in entoderm to form blood cell.

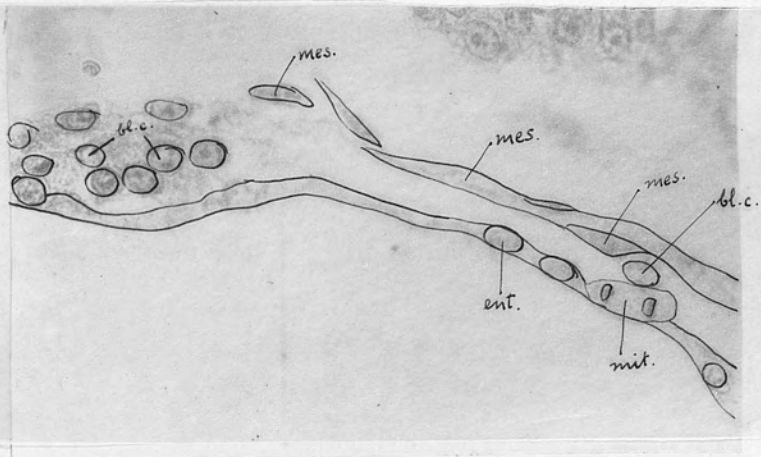
FIGURE 16 X 500.

BLOOD CELLS PARTIALLY ENGULFED
BY ENDOTHELIUM.



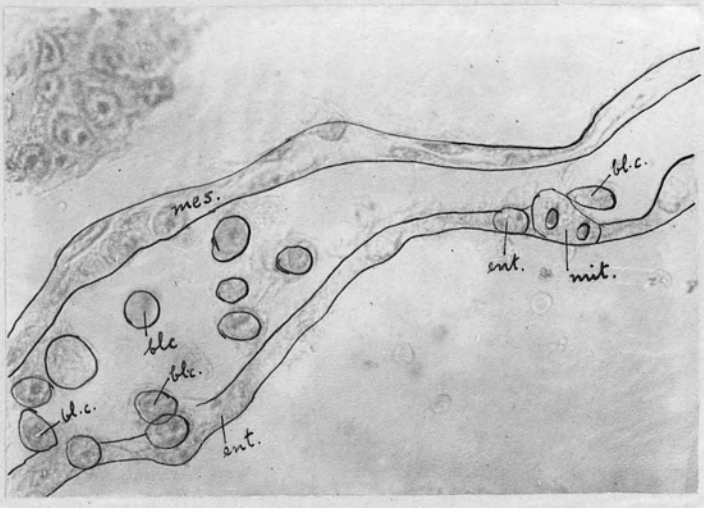
ent. - Entoderm.
mes. - Mesoderm.
bl.c. - Blood cells.
end. - Endothelium.

FIGURE 17a. X 500.



ent. - Entoderm.
mes. - Mesoderm.
bl.c. - Blood cells.
mit. - Mitosis in entoderm to form blood cell.

FIGURE 17b. X 500.



Same as in Fig. 17a.

STAGE III.

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The material for this stage consists of one embryo which is 2.3mm. in length and with 9 somites.

DESCRIPTION OF THE GRAPHIC RECONSTRUCTION OF THE HEART AND THE CRANIAL PORTION OF A FERRET EMBRYO 2.3mm. IN LENGTH WITH 9 SOMITES. (F.Ap. 16/28/08.).

General Description.

The amnion is closed caudally. The allantoic diverticulum and the allantoic mesoderm are both present. The neural groove extends to the caudal end of the embryo, but terminates, however, at some distance cranial to the tail amnion-fold. It narrows and deepens as it passes cranially.

The rudiment of the otic ganglion is distinct and the rudiment of the trigeminal ganglion is likewise recognizable.

The head-fold has begun to form and a portion of the fore-gut is also defined.

There is no definition of the primary optic vesicle.

Vascular System.

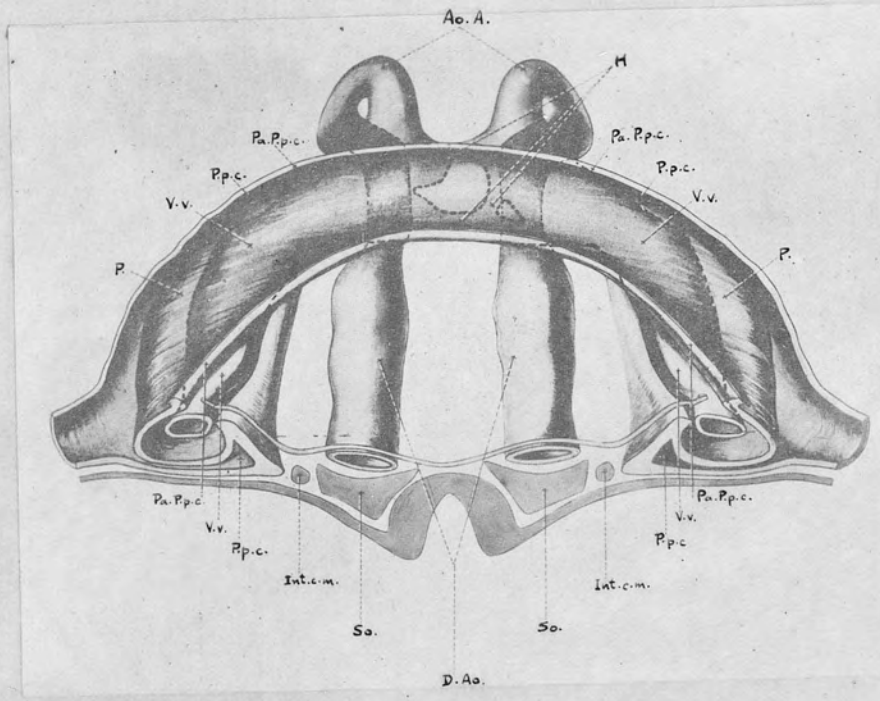
The heart of this embryo has not yet been reconstructed in wax, but the graphic reconstruction (Fig. 18.) which has been made shows that, in length, this specimen is identical with the Stage IIb. embryo, but in its general development it is decidedly in a more advanced stage than the preceding one, as the central part of the transverse rudiment of the heart seen in Stage II (fig. 6a.) has, in this specimen, begun to break up, two non-vascular loculi having divided it incompletely into a cranial and a caudal portion (figs. 18, 19a, a', b, b', c, c', d, d', e, and e'). Apparently the division of the central part of the rudimentary transverse heart proceeds still further as development goes on, until it is completely separated into right and left halves, for in Stage IV (fig. 27) the heart rudiment is represented by two separate longitudinal endothelial tubes which lie side by side and are in contact in their middle third.

Cranially two vessels, one on each side of the median plane, run cranialward from the heart rudiment. They arch round the cranial end of the fore-gut and form the first cephalo-aortic arches, which terminate dorsally in the corresponding dorsal aortae (Figs. 18 and 20).

FIGURE 18 X 100.

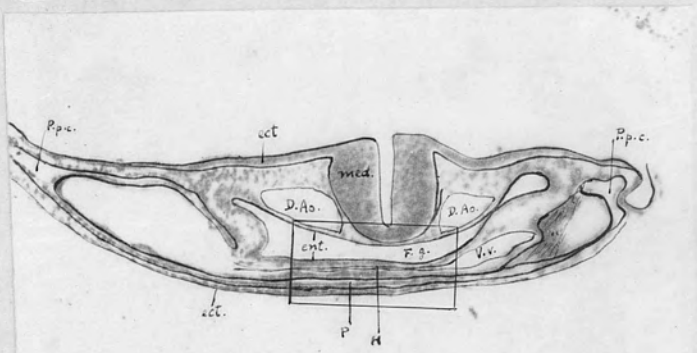
SEMI - SCHEMATIC.

******



D.Ao.	-	Dorsal aorta.
Ao.A.	-	Aortic arch.
H.	-	Heart rudiment breaking up.
P.	-	Pericardium.
P.p.c.	-	Pleuro-pericardial canal.
Pa.P.p.c.	-	Cut edge of parietal layer of Pleuro-pericardial canal.
V.v.	-	Vitelline vein.
So.	-	Somite.
Int.c.m.	-	Intermediate cell mass.

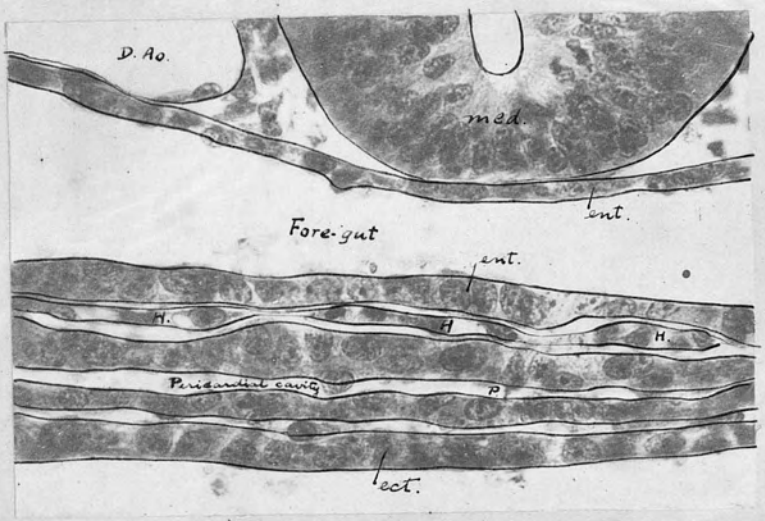
FIGURE 19a X 100.



D.Ao. - Dorsal aorta.
H. - Heart rudiment united across.
P. - Pericardium.
P.p.c. - Pleuro-pericardial canal.
V.v. - Vitelline vein.
F.g. - Fore-gut.
ent. - Entoderm.
ect. - Ectoderm.
med. - Medulla.

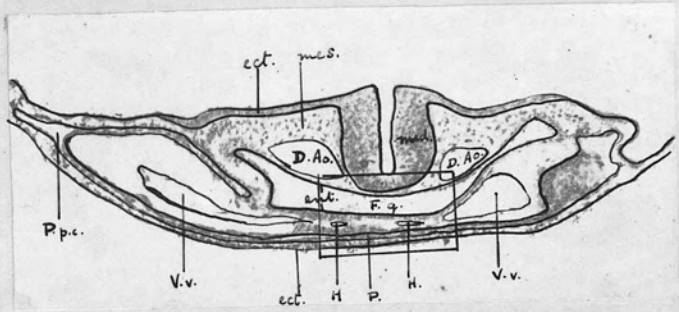
FIGURE 19a' X 500.

(Magnified from the Square in Fig. 19a)



D.Ao. - Dorsal aorta.
H. - Heart rudiment united across.
P. - Pericardium.
F.g. - Fore-gut.
ent. - Entoderm.
ect. - Ectoderm.
mes. - Mesoderm.

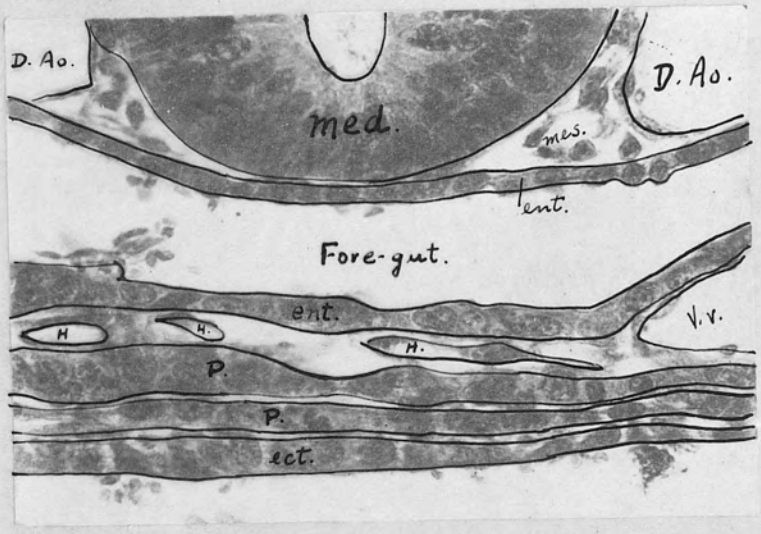
FIGURE 19b. X 100.



D.Ao. - Dorsal aorta.
H. - Heart rudiment broken up.
P. - Pericardium.
P.p.c. - Pleuro-pericardial canal.
V.v. - Vitelline vein.
F.g. - Fore-gut.
med. - Medulla.
ect. - Ectoderm.
ent. - Entoderm.
mes. - mesoderm.

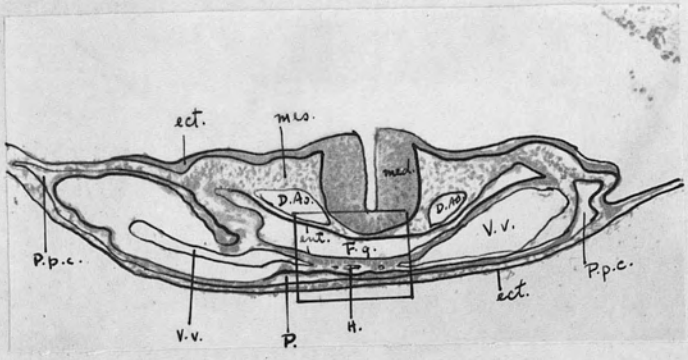
FIGURE 19b' X 500.

(Magnified from the Square in Fig. 19b)



Same as in Fig. 19b.

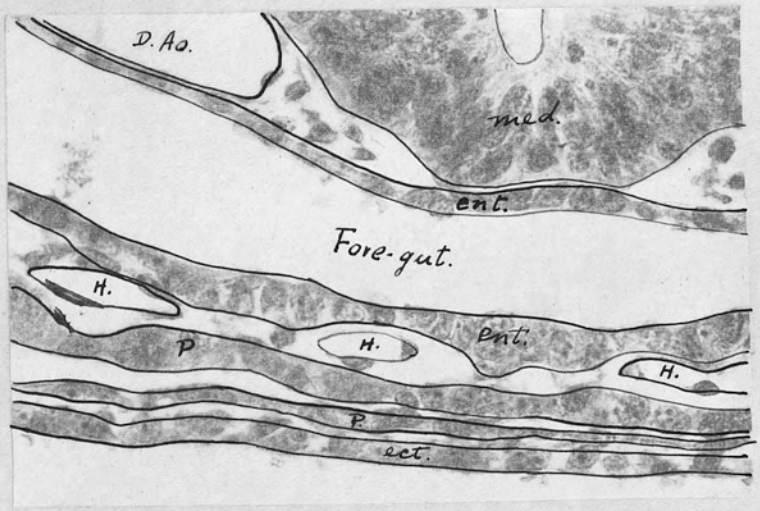
FIGURE 19c. X 100.



Same as in Fig. 19b.

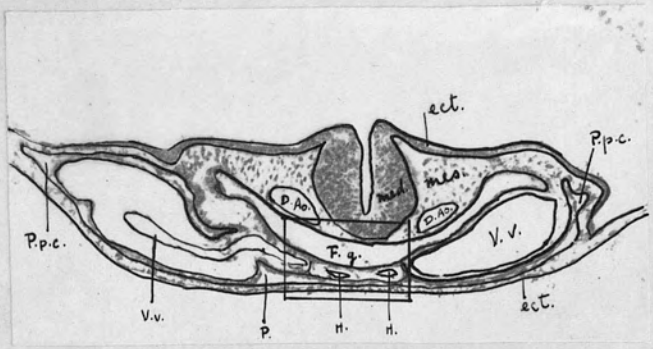
FIGURE 19c' X 500.

(Magnified from the Square in Fig. 19c)



Same as in Fig. 19b'.

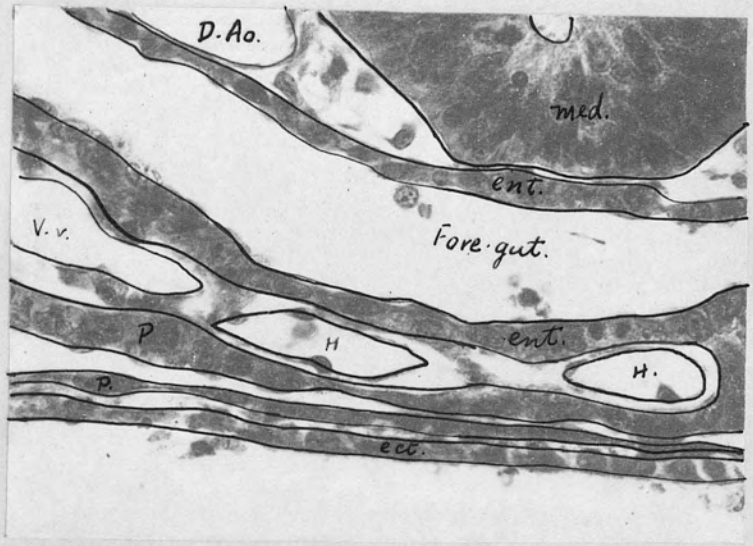
FIGURE 19d. X 100.



Same as in Fig. 19c.

FIGURE 19d' X 500.

(Magnified from the Square in Fig. 19d)

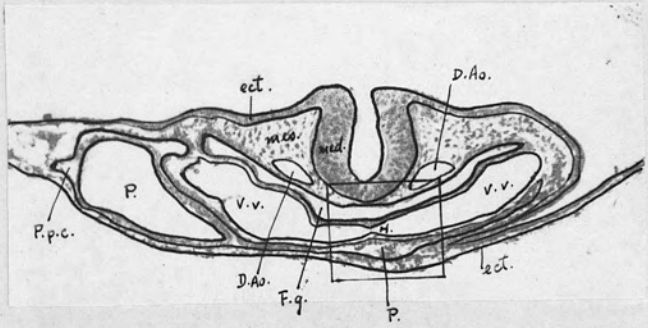


Same as in Fig. 19c',

FIGURE 19e. X 100.

HEART RUDIMENT UNITED ACROSS.

*****



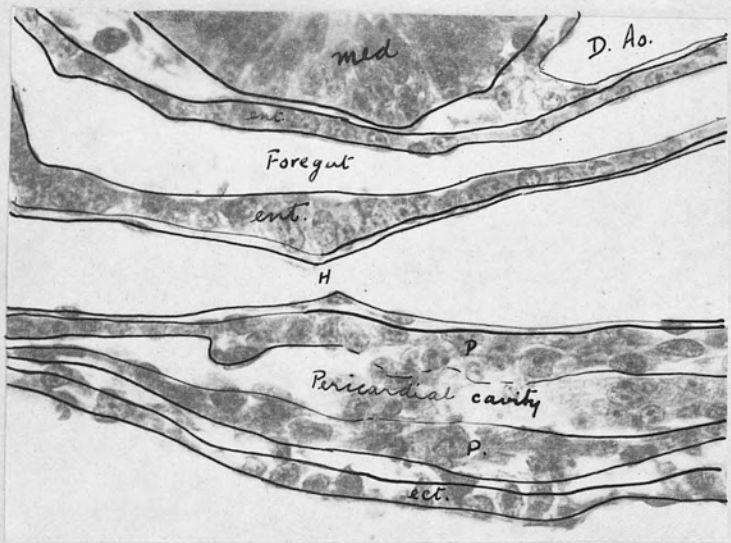
Same as in Fig. 19a.

FIGURE 19e' X 500.

(Magnified from the Square in Fig. 19e)

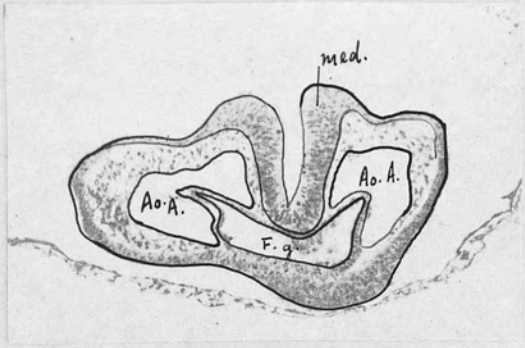
HEART RUDIMENT UNITED ACROSS.

*****



Same as in Fig. 19e.

FIGURE 20 X 100.



Ao.A. - Aortic arch.
F.g. - Fore-gut.
med. - Medulla.

(Figs. 18 and 20). Caudally the heart rudiment receives the two vitelline veins. It is obvious that the fore-gut has begun to develop pari passu with the head-fold.

The pericardial cavity is much wider and longer than it is in the preceding specimen, measuring 870 μ in the transverse diameter and 140 μ in the antero-posterior direction. The two dorsal aortae are well developed and run parallel to each other one on each side of the median plane of the embryo not far from the medullary groove (Figs. 18, and 19a-e). Here again, there is nothing to indicate the presence of a ventral mesocardium.

This stage of development, as far as the heart is concerned, appears to fall in between the Stage II Dasyurus viverrinus (8.5mm.) and the Stage III Perameles nasuta (7.5mm., 11 somites) of Miss Parker(15). In the Dasyurus the endothelial tubes have come actually in contact with each other at their extreme cranial ends and presumably have united across the median plane of the embryo. It should be noted that the significance of this connection between the two vitelline veins across the median plane was not dwelt upon and was considered only as being remarkable by Miss Parker, who states also that lateral and caudal to the median union, each

endothelial

endothelial tube gives rise to the first aortic arch, which follows the antero-lateral margin of the gut almost to the median plane and there becomes continuous with the corresponding dorsal aorta, the two aortae being well-developed at this stage.

It is quite probable that what has been taken for the first aortic arch in Miss Parker's Dasyurus specimen, as in the case of Eternod's human embryo (vide p. 21), may, after all, prove to be the plexus which lies between the dorsal aorta and the vitelline vein and which has been described by Bremer(12) to be present in the rabbit embryo of 5 somites. If this is the case, the Stage II Dasyurus may be regarded as being similar with the Stage IIa ferret embryo, in so far as the development of the heart, the vitelline veins and the dorsal aortae is concerned.

In the Perameles (7.5mm.), the two heart tubes lie separate from each other ventral to the closed fore-gut. At the level of the umbilical orifice they diverge and lie on each side of the open gut in the dorso-medial wall of the pericardium. From the cranial extremity of each endothelial tube, there arise two vessels, one of which run cranially and laterally towards the lateral margin of the gut and then parallel with this margin. It loops round the

cranial

cranial end of the gut, joins the dorsal aorta, and thus constitutes the first aortic arch. The other vessel is small and runs caudally and laterally, lateral to and almost parallel with the heart tube. This, according to Miss Parker, is the ventral portion of the future second aortic arch. In the median space between the cranial ends of the endothelial heart tubes are a number of scattered angioblast cells lying between the splanchnic mesoderm and the entoderm.

STAGE IV.

***

This stage is represented by one embryo 2.5mm. in length, the cranial extremity of which has been reconstructed in wax.

DESCRIPTION OF THE PLASTIC RECONSTRUCTION OF THE HEART AND THE CRANIAL PORTION OF A FERRET EMBRYO 2.5mm. IN LENGTH WITH 12 PAIRED SOMITES. (F.Ap. 13/28/08).

A plastic reconstruction of the cranial portion of this embryo has been made. It is therefore possible to present a fuller description of the head region of the specimen.

The Reconstruction of the embryo was exhibited, along with several other Reconstructions of older specimens, before the Anatomical Section of the International Medical Congress at its meetings held in London in August 1913.

TECHNIQUE.

*****

The mother was killed with chloroform and the abdomen opened immediately. The whole uterus was removed and the embryo fixed en bloc with warm Zenker's fluid. It was afterwards washed in running

water

water and transferred to industrial spirit coloured with iodine in the usual manner.

The embryo, with about half of the uterine wall attached to it, was stained in bulk with Meyer's acid haemalum, and counter-stained with eosin. After subsequent treatment in the usual way, the specimen was embedded in paraffin, trimmed and provided with guiding-lines. The embryo, which was exposed to view after the dorsal half of the uterine wall was removed, was carefully measured before being fixed and stained, and was found to be 2.5mm. in length, age being 14 days old approximately. It was then cut in serial sections with a thickness of 10 microns and yielded 240 perfect sections, there being, therefore, a slight but uniform shrinkage of .1mm. The plane of section was fairly horizontal and almost transverse to the long axis of the embryo in caudo-cranial succession.

On microscopical examination the resulting sections are found to be of excellent quality and present a perfect histological picture with frequent mitotic figures and a normal condition of the general contour of the epithelial linings of the various organs, vessels and body spaces - all pointing to the specimen being normal.

Tracings of every section of the cranial end of the embryo were drawn with the aid of the

projector

projector apparatus at a magnification of 100 diameters, and these, in turn, were made into wax-plates of 1.mm. in thickness. When the plates were cut out and methodically adjusted into position with careful manipulation of the guiding-lines, and in their numerical order, it was found that they superimposed one another most accurately, and that the structures faithfully took up their relative anatomical positions. After the model of the embryo had thus been built up, the original guiding-lines were dispensed with, but, to minimize any error that might arise in the subsequent divisions of the Reconstruction into detachable blocks to expose to view the deeper organs, new guiding-lines were made on the surface of the Reconstruction. The plates were then solidified and slightly smoothed, and the structures painted over with different colours to represent the various organs. The model is now to be seen at the Anatomical Department, Edinburgh University.

Such plastic reconstructions, generally known as Born's Reconstruction, as has been often pointed out, are not to be considered as absolutely free from error even with the most accurate manipulation of the wax-plates provided with the most reliable guiding-lines; for it has been noticed that the variations of temperature in the room at the time when the model is under the process of reconstruction, or even after the

the reconstruction is completed, materially alter, though in a small degree, the consistency of the wax employed, and therefore affect the model; but the results obtained with the Born's method are such as cannot be so conveniently produced by any other known method.

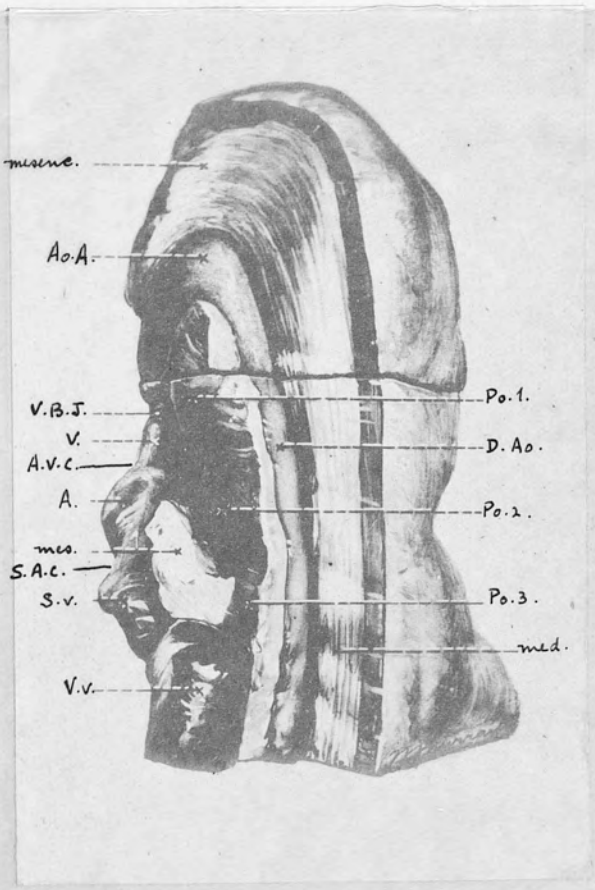
General Description.

It should be pointed out that the embryo under consideration came from the same uterus as the one of 13 somites described by Yeates(15) to which references will be made hereafter.

The embryo is, in certain ways, similar to the human embryo described by Alexander Low(08), which measures 2.6mm. in length and exhibits 13 - 14 somites. Three visceral pouches can be distinguished as in the case of Yeates'(15) specimen, but there are only two external clefts in the specimen (Figs. 21. and 25.). In the latter respect the ferret embryo may be regarded as being somewhat younger than the one described by Low, which shows three visceral clefts besides possessing an S-shaped heart tube. The brain flexure of the ferret embryo at this stage corresponds, in a measure, more to the ferret embryo of 13 somites described by Yeates and to the human embryo of 13 somites described by Ivan E. Wallin(13)

than

FIGURE 21. X 100.



Po1.	-	First Visceral Pouch.
Po2.	-	Second Visceral Pouch.
Po3.	-	Third Visceral Pouch.
V.v.	-	Vitelline vein.
S.v.	-	Sinus venosus.
S.A.C.	-	Sino-Atrial canal.
A.	-	Atrium.
A.V.C.	-	Atrio-Ventricular canal.
V.	-	Ventricle.
V.B.J.	-	Junction between Ventricle & Bulbus Cordis.
Ao.A.	-	Aortic arch.
D.Ao.	-	Dorsal aorta.
mes.	-	Cut surface of Mesodermic tissue
mesenc.	-	Mesencephalon.
med.	-	Medulla.

than to the one described by Low.

As in all of the embryos above quoted, but resembling more closely to the 4.mm. human embryo described by Bremer(05-06), the medullary tube opens to the exterior at its cranial and caudal extremities, the former(Figs. 22, 25, and 27) with a slit-like but bent aperture over a distance of 28 sections, and the latter with an opening of equal length. This feature harmonizes with the statement made in Keibel's Normentafeln, that in both the pig and the rabbit embryos, the closure of the medullary tube is completed only after the formation of the head and neck bends. It may be mentioned here that the bucco-pharyngeal membrane is present(Figs. 22 and 27), and that the rudiment of the Wolffian body is first seen opposite the 7th. somite and thereafter it is not separated from the paraxial and lateral mesoderm.

SOMITES.

There are to be found twelve pairs of well-formed somites which have been determined by careful counting under the microscope. The first formed somite is distinctly caudal to the otic- plate by a distance of .2mm. As the ganglia are not yet well

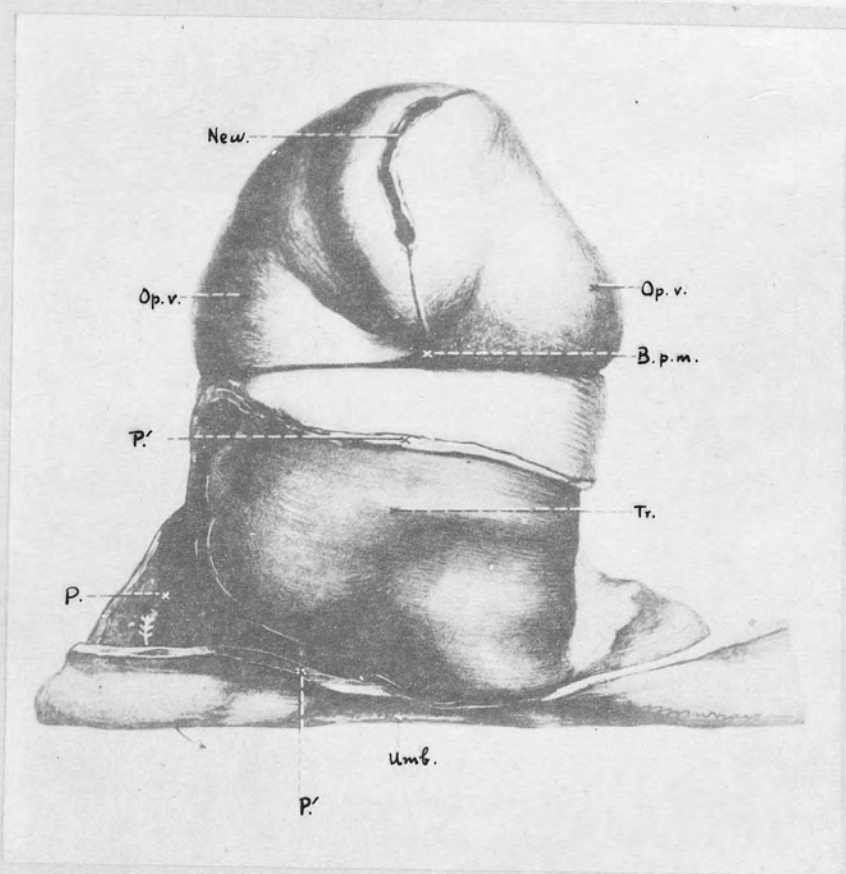
developed

developed, it is futile to attempt, at this stage of development, to allot the different somites to their respective regions.. Each somite has a uniform thick wall three or four cells in depth, enclosing a cavity (myocoel) which looks very distinct in the more caudal somites, but less so in those situated more cranially. Many of the nuclei, which lie near the coelom of the somite, show definite signs of mitosis. The more cranially placed somites (Fig. 23) are distinctly triangular in shape on cross section with the apices pointing ventro-medially and the bases dorso-laterally, while the more caudal ones (Fig.24) are, more or less, quadrangular in outline.

ALLANTOIS.

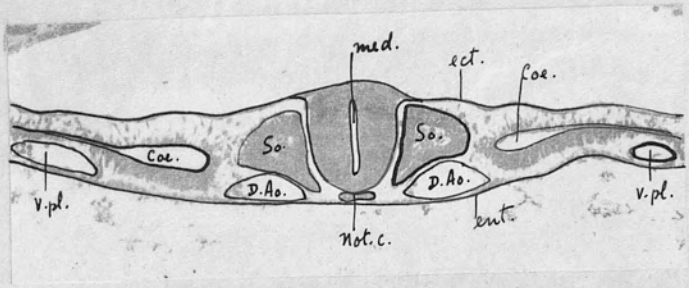
The allantois may be described as having the appearance of a small diverticulum on the caudal end of the entodermal sac. Its caudal extremity is, however, bifid, as noted by Yeates of Birmingham(11); the bifidity includes both the entodermal and mesodermal components. This mode of development of the allantoic cavity seems to occupy an intermediate position between that of the typical mammal and that of the lizard.

FIGURE 22 X 100.



Neu. - Neuropore.
B.p.m.- Bucco-pharyngeal membrane.
P. - Pericardium.
P'. - Cut surface of Pericardium.
Tr. - Transverse connection between lateral segments of S-Shaped muscular heart tube.
Umb. - Umbilical orifice.
Op.v. - Optic vesicle.
Arrow in Pleuro-pericardial canal.

FIGURE 23. X 100.



med. - Medullary tube.
So. - Somite.
Coe. - Coelom.
D.Ao. - Dorsal aorta.
V.pl. - Vitelline plexus.
Not.c. - Notochord.
ect. - Ectoderm.
ent. - Entoderm.

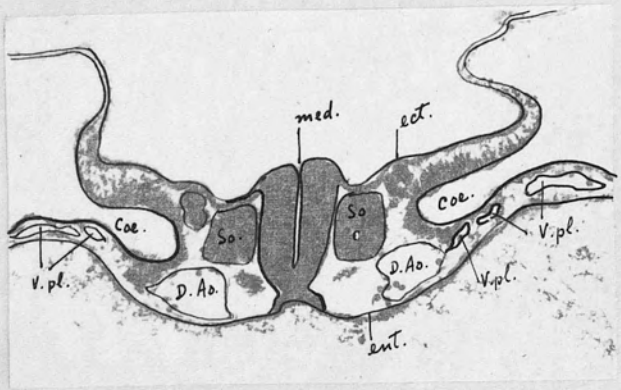
NERVOUS SYSTEM.

The medullary tube, as already pointed out, is closed except at its cranial and caudal ends, where the cranial and caudal neuroporic apertures are present. The mesencephalon is flexed upon itself, in such a way as to bring the prosencephalon almost to a plane parallel with the long axis of the rhombencephalon which seemingly merges, without any appreciable demarcation, into the spinal medullary tube (Fig. 25.). The pontine and cervical flexures are definitely absent in this specimen. The otic vesicle is represented only by a thickened plate of epithelium which shows no indication of invagination, and is situated .2mm. cranial to the first pair of somites (vide supra). There is yet no lens thickening to be detected. The primary optic vesicle on each side is, however, well represented by a slight bulge of the lateral wall of the anterior portion of the prosencephalon of the corresponding side, much in the same way as in the specimen described by Yeates (15).

When the whole series of the sections is examined under the high power of the microscope, the notochord is found to be free throughout its whole course, except at the cranial part, where it is still

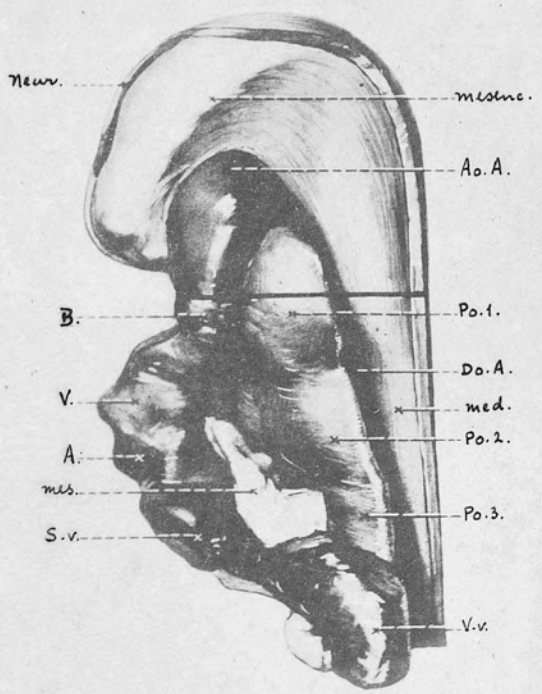
connected

FIGURE 24. X 100.



med. - Medullary tube.
So. - Somite.
D.Ao. - Dorsal aorta.
V.pl. - Vitelline plexus.
Coe. - Coelom.
ect. - Ectoderm.
ent. - Entoderm.

FIGURE 25. X 100.



Po1.	-	First Visceral Pouch.
Po2.	-	Second Visceral Pouch.
Po3.	-	Third Visceral Pouch.
V.v.	-	Vitelline vein.
S.v.	-	Sinus venosùs.
A.	-	Atrium.
V.	-	Ventricle.
B.	-	Bulbus.
Ao.A.	-	Aortic arch.
D.Ao.	-	Dorsal aorta.
mes.	-	Cut surface of Mesodermic tissue
med.	-	Medulla.
mesenc	-	Mesencephalon.
Neur.	-	Neuropore.

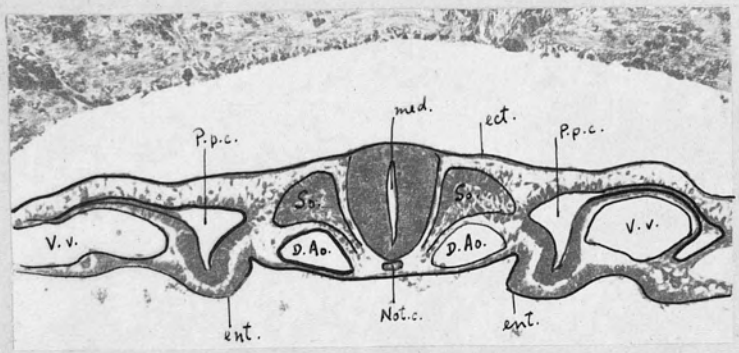
connected with the gut wall from a point opposite the first somite to the bucco-pharyngeal membrane, and at the caudal end, where it is connected with the entoderm from the level of the 7th. somite to the end of the body of the embryo. The notochordal canal at this stage of development, as described by Mall(12), Eternod(95, 99), and Grosser(13), is just discernible under the high power (Fig. 26). In the ferret embryo of 13 somites it is to be noted, no lumen could be made out by Yeates(15) in either the cranial or the caudal parts of the notochord. In places the cells of the notochord are arranged in two lateral masses which suggest the presence of bilateral symmetry (Fig. 23). The cells of the chorda are comparatively large, oval and clear.

PERICARDIUM.

The Reconstruction shows that at this stage of development the pericardial cavity is found to be closed except in its caudo-lateral aspect, where it communicates, on each side, with the pleuro-pericardial canals which are situated dorso-medial to the vitelline veins (Figs. 22 and 30). The pericardium, measuring 875 μ in width and 350 μ in length, is reflected on to the two endothelial tubes on its

dorsal

FIGURE 26. X 100.



med. - Medullary tube.
So. - Somite.
Not.c. - Notochord.
D.Ao. - Dorsal aorta.
V.v. - Vitelline vein.
P.p.c. - Pleuro-pericardial canal.
ect. - Ectoderm.
ent. - Entoderm.

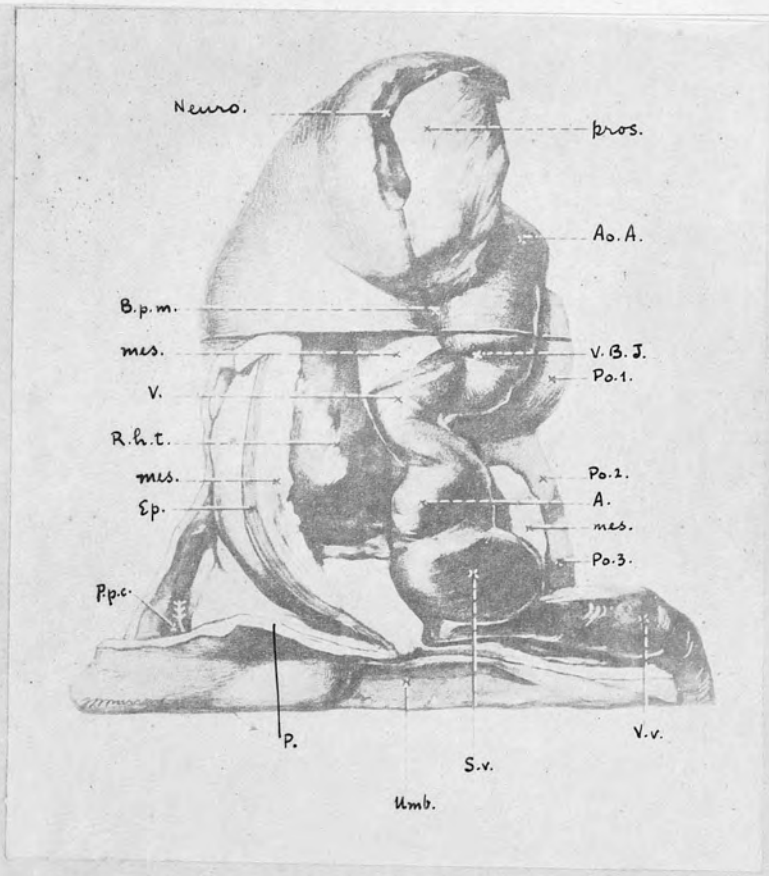
dorsal aspect forming the dorsal mesocardium, but ventrally, there is not even the vestige of the ventral mesocardium, which, in birds and amphibians, is so conspicuously seen at this stage of development.

HEART.

With the ventral portion of the pericardium removed (Fig. 22), it is to be observed that the ventral aspect of the heart rudiment is separated in the caudal but larger portion of its extent into a right and left segment by a cranio-caudal sulcus. On each lateral half, there are also several transverse sulci, but the significance of these various sulci is rather obscure, as the segments marked out by them do not correspond, in any way, with the primary divisions of the heart tube, although at first sight, it appears that the left segment is apparently atrial, and the right, ventricular. The fact that this appearance is deceptive becomes at once obvious when a portion of the muscular wall of the heart tube is removed (Fig. 27); for it is then seen that within the lumen of the muscular tube, and separated from its wall by a more or less thick mass of loose mesodermic tissue, lie two endothelial tubes, which are quite separate from one another in the

whole

FIGURE 27. X 100.



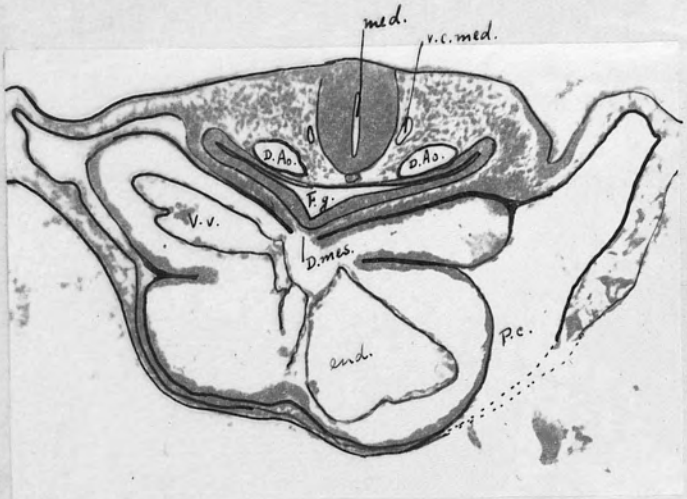
Po1.	-	First Visceral Pouch.
Po2.	-	Second Visceral Pouch.
Po3.	-	Third Visceral Pouch.
V.v.	-	Vitelline vein.
S.v.	-	Sinus venosus.
A.	-	Atrium.
V.	-	Ventricle.
V.B.J.	-	Junction between Ventricle & Bulbus Cordis.
Ao.A.	-	Aortic arch.
mes.	-	Cut surface of Mesodermic tissue
pros.	-	Prosencephalon.
R.h.t.	-	Right heart tube.
Neuro	-	Neuropore.
Ep.	-	Epicardium.
P.	-	Pericardium.
B.p.m.	-	Bucco-pharyngeal membrane.
Umb.	-	Umbilical orifice.
Arrow	in	Pleuro-pericardial canal.(P.p.c.)

whole of their extent (Figs. 27, and 28a-d). Each endothelial tube can be followed caudally to the septum transversum, where it is continuous with the corresponding vitelline vein immediately cranial to the umbilical orifice (Figs. 27 and 28a), and cranially it is continuous with the first cephalo-aortic arch, which runs dorsally through the mandibular arch round the cranial border of the first visceral pouch (Figs. 25, 27 and 29).

In the Reconstruction (Fig. 27) the muscular coat of the heart rudiment has been removed entirely on the left side but only partly on the right side. In this way the left endothelial tube is, therefore, fully exposed to view on its ventral and lateral aspects, and it exhibits clearly a very definite indication of separation into divisions which appear to indicate, in caudo-cranial succession, the positions of the sinus venosus, the sino-atrial canal, the atrium, the atrio-ventricular canal, the ventricle and the bulbus cordis (Fig. 27).

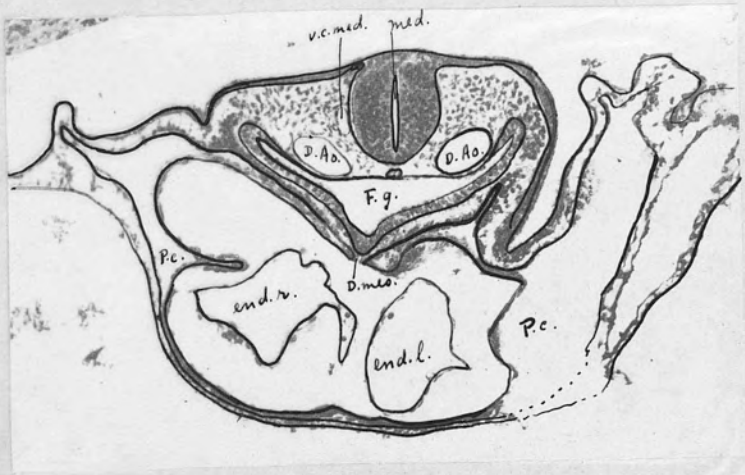
The dilatation (Figs. 25 and 27) which represents the sinus venosus occupies the most caudal portion of the tube and is partly embedded in the substance of the septum transversum and partly projects into the pericardial cavity. At its caudal end near its dorsal aspect, it receives the corresponding

FIGURE 28a. X 100.



med. - Medullary tube.
F.g. - Fore-gut.
D.mes. - Dorsal mesocardium.
D.Ao. - Dorsal aorta.
P.c. - Pericardial cavity.
V.v. - Vitelline vein.
end. - Left endothelial tube.
v.c.med- Vena capitis medialis.

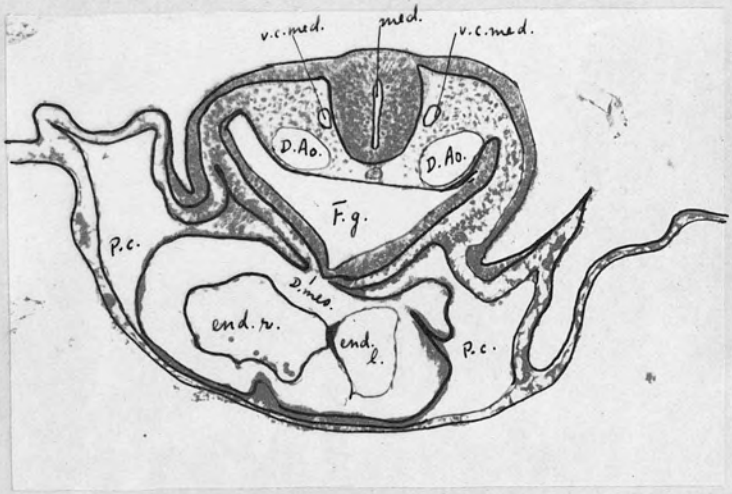
FIGURE 28b. X 100.



med. - Medullary tube.
F.g. - Fore-gut.
D.mes. - Dorsal mesocardium.
D.Ao. - Dorsal aorta.
P.c. - Pericardial cavity.
end.r. - Right endothelial tube.
end.l. - Left endothelial tube.
v.c.med.- Vena capitis medialis.

FIGURE 28c. X 100.

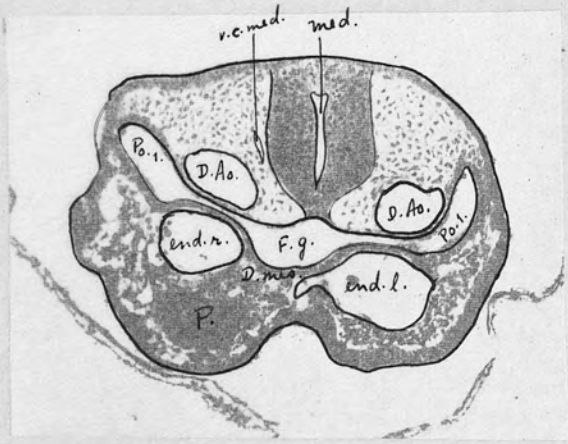
RIGHT AND LEFT ENDOTHELIAL
TUBES IN CONTACT BUT NOT FUSED.



med. - Medullary tube.
F.g. - Fore-gut.
D.mes. - Dorsal mesocardium.
D.Ao. - Dorsal aorta.
P.c. - Pericardial cavity.
end.r. - Right endothelial tube.
end.l. - Left endothelial tube.
v.c.med.- Vena capitis medialis.

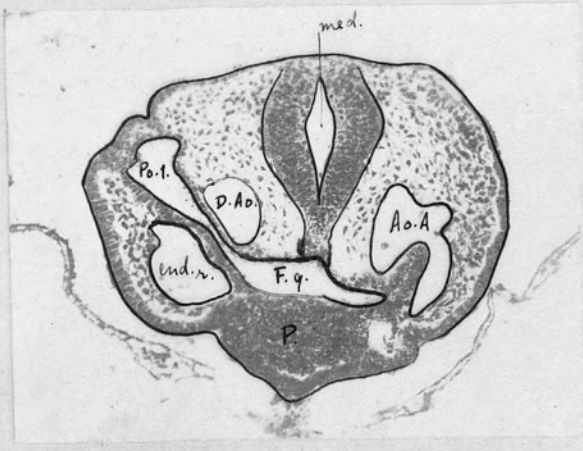
FIGURE 28d. X. 100.

RIGHT AND LEFT ENDOTHELIAL TUBES EMERGING FROM PERICARDIAL CAVITY CRANIALLY.



med. - Medullary tube
F.g. - Fore-gut.
Po1. - First Visceral Pouch.
D.mes. - Dorsal mesocardium.
D.Ao. - Dorsal aorta.
P.c. - Cut surface of cranial pericardial reflection.
end.r. - Right endothelial tube
end.l. - Left endothelial tube.
v.c.med.- Vena capitis medialis.

FIGURE 29. X 100.



med. - Medullary tube.
F.g. - Fore-gut.
Po1. - First Visceral Pouch.
D.Ao. - Dorsal aorta.
Ao.A. - Aortic arch.
P. - Cut surface of cranial pericardial reflection.
end.r. - Right endothelial tube.

corresponding vitelline vein, which turns abruptly from the transverse to a caudo-cranial direction before it empties itself into the sinus venosus (Fig. 27).

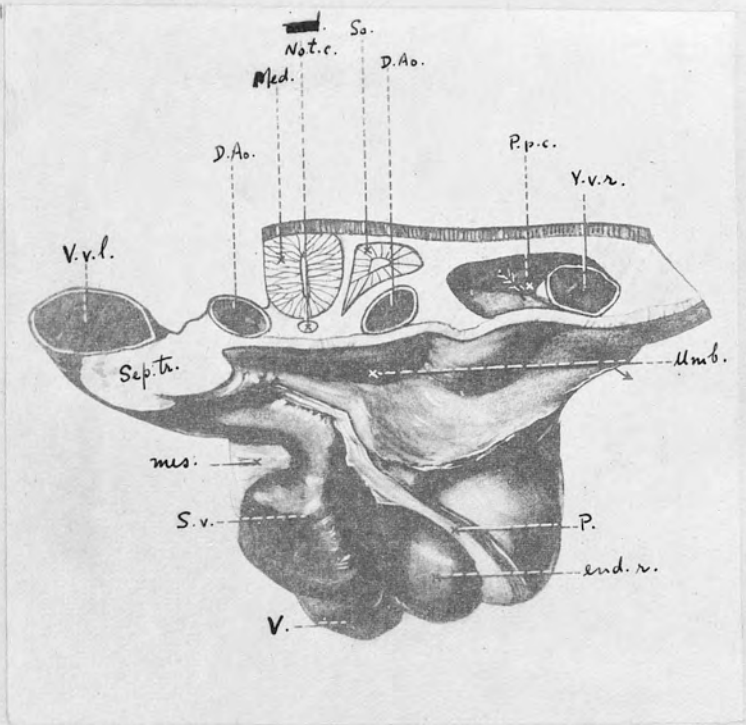
The constriction which indicates the position of the sino-atrial canal is distinctly marked laterally and dorsally (Fig. 27). It is less conspicuous ventrally, and is altogether absent on the medial side of the tube. The dilatation (Figs. 25 and 27) which takes the place of the atrium is comparatively small, and the constriction (Fig.27) which indicates the position of the atrio-ventricular canal can easily be recognized on the medial and ventral aspects of the tube and slightly on the dorsal, but there is nothing to suggest a constriction on the lateral aspect in this situation (Fig. 25).

The ventricle which is situated at the most cranial end of the endothelial tube, is bent upon itself (Fig. 27), and the most dependent point of the tube is the most ventral part of the ventricular portion (Figs. 25 and 30). It is divided by a shallow depression, clearly recognizable on the lateral aspect (Fig. 27), into a caudal and a cranial limb; the latter is separated by a somewhat oblique constriction from the bulbus cordis, which is bulged laterally and then turns cranially to be continuous

with

FIGURE 30. X 100.

CAUDAL VIEW.



med. - Medullary tube.
So. - Somite.
D.Ao. - Dorsal aorta.
Not.c. - Notochord.
Umb. - Umbilical orifice
P.p.c. - Pleuro-pericardial canal.
mes. - Cut surface of mesodermic tissue.
P. - Cut surface of Pericardium.
V.v.l. - Left Vitelline vein.
V.v.r. - Right Vitelline vein.
Sep.tr. - Septum transversum.
S.v. - Sinus venosus.
end.r. - Right endothelial tube partially exposed.
Arrow in Pleuro-pericardial canal.
V. - Ventricle.

with the ventral aorta immediately below the caudal extremity of the ventral part of the first visceral pouch.

The right endothelial tube, which has not been fully exposed in the Reconstruction, appears to present similar dilatations and constrictions, the outlines of which can be followed, to a certain extent, through the muscular covering, but, since in the model it is still covered in parts by muscular substance, the exact details cannot be worked out at present, and therefore no positive statement regarding it can be put forward. It is, however, more dorsally situated than the left tube (Fig. 30); moreover, the whole heart is bent slightly towards the right side in the ventricular region (Fig. 27).

The heart at this stage of development is attached to the dorsal wall of the pericardium (Figs. 28a-d). There is positively no trace of a ventral mesocardium in the ferret embryo (a point already alluded to, but to be fully discussed later), and the dorso-ventral length of the dorsal mesocardium is extremely short. The heart is applied closely to the ventral aspect of the pharynx in the region of the second visceral pouch.

In this communication it is proposed not to enter in detail into the development of the blood

vessels

vessels beyond the region of the heart, but, in passing, it is perhaps of interest to note that the ventral aorta communicates from the dorsal end of the bulbus cordis and runs cranially but ventral to the first visceral pouch, then turns dorsally round the cranial border of the pharynx to form the first cephalo-aortic arch, and, finally, it pursues a course caudally along the dorsal wall of the fore-gut as the dorsal aorta (Figs. 21 and 25). It is a relatively wide vessel, its calibre throughout being distinctly greater than that of the bulbus cordis.

Immediately dorsal to the dorsal aorta there is situated on each side of the embryo a series of apparently isolated sections of a minute blood vessel (Figs. 28a-d). These capillaries lie close against the medullary tube. This vessel appears to be the vena capitis medialis of Grosser(95), which Miss Parker also has found to be present in her Stage III Perameles nasuta 7.5mm.

Intersegmental off-shoots from the dorsal aortae in the region of the caudal somites are described by Miss Parker in Perameles nasuta, but no such off-shoots from the dorsal aortae could be found in the ferret embryo at this stage. In the caudal region of the ferret embryo, the two dorsal

aortae

aortae become continuous with the vitelline arteries, which spread themselves out in a plexiform manner on the wall of the yolk-sac (Fig. 24.). This stage of development of the dorsal aorta in the ferret agrees, in some ways, with the 1.3mm. human embryo described by Eternod (95, 99).

The vitelline vein (Fig. 27) which opens into the caudal end of the sinus venosus, so far as it lies on the embryonic region, runs at first transversely towards the median plane in the substance of the septum transversum immediately cranial to the umbilical orifice. As it approaches the median plane, it changes its course suddenly, making a sharp bend upon itself cranially, and terminates, as has already been indicated, in the dorsal extremity of the caudal part of the corresponding sinus venosus. It is applied so closely to the margin of the umbilical orifice that its caudal border causes a distinct bulging of the boundary of the orifice (Fig. 27).

For the sake of comparison, and for the purpose of bringing out the chief differences, as far as the development of the heart is concerned, between this specimen and that of Yeates(15), the description of the endothelial tubes of the latter given by Yeates might be quoted:

"As the endothelial tubes course through the primitive cavity of the heart (the primitive cavity

of

of the heart means the cavity within the muscular tube - the myoepicardium) they are separated by a variable but distinct interval from the myoepicardium. The myoepicardial tube has previously been stated to present constrictions at each extremity, at the sinuatrial junction and at the atrio-ventricular canal. The endothelial tubes present corresponding constrictions. In the region of these constrictions the endothelial tubes are relatively close to the primitive myoepicardium, whilst they gradually recede from the heart wall as the middle of each of the three primitive cavities of the muscular heart is approached. In other words, the muscular cavity is more expended, and the endothelial is more tubular between the constrictions. The endothelial tubes are in contact medianly in the cranial two-thirds of the atrium, in the ventricle and in the region of the bulbus. In the atrio-ventricular canal they are not only in contact but have fused and are partially absorbed, so that their cavities communicate across the median plane. On the other hand, in the region of the sinus venosus and of the truncus, the tubes are free and separate from each other. The portions of the tubes which are in contact within the atrium are connected by delicate endothelial strands with the inner aspect

of

of the ventral wall of the atrium along the crest of the irregular ridge, which has been spoken of as the remains of the primitive cardiac septum. The ventricular limb of the endothelial tubes are subdivided into ventricle, bulbus cordis, and truncus arteriosus, by two faintly marked constrictions. The endothelial heart, therefore, consists of, not only sinus venosus, atrium, and ventricle, as in the muscular heart, but also of bulbus cordis and truncus arteriosus."

It will be noticed then that the specimen just cited is decidedly in a more advanced stage of development, since the two endothelial tubes have partially fused in the region of the atrio-ventricular canal, much in the same way as the Stage V specimen to be presently described.

Subdivisions of both of the endothelial tubes into sinus venosus, atrium, ventricle, bulbus cordis and truncus arteriosus have been observed also by Yeates in his specimen; but unlike those of the Stage IV 2.5mm ferret embryo these subdivisions correspond in the main in positions to those exhibited in the muscular (myoepicardial) wall of the heart tube. One point which is clear is that the endothelial tubes differentiate into their various subdivisions more completely than the muscular tube.

It should be pointed out that the second aortic

arch

arch in Yeates' specimen has already made its appearance.

It is to be noted that, as far as the external appearance of the heart is concermed, the ferret embryo at this stage of development shows certain prominent features which are in many respects identical with those of the dog of a similar stage of development, and which have been investigated by Bonnet(01). In his papers it has not been possible to find a comprehensive description of the development of the heart of the dog. In figure vi (Anat. Heft. 1901. Bd. 16) Bonnet depicted a dog embryo 5.7mm. long with 10 pairs of somites. Here the heart is represented by two endothelial tubes which are distinctly separated from one another and are both bent towards the right side in the ventricular region. Each endothelial tube has, for its caudal continuity, the corresponding vitelline vein, which runs latero-medially and at the same time cranially towards the sinus venosus. There seems to be no distinctive demarcation between the sinus venosus and the atrium, but between the atrium and the ventricle there is a constriction both on the lateral side and medial side. The ventricle looks very much dilated in the figure, so much so that, when viewed from its ventral aspect, the whole heart appears to

be

be formed by the distended ventricle, with the bulbus cordis and atrium attached to it cranially and caudally respectively as mere appendages. The bulbus cordis is clearly constricted off from the ventricle, and its calibre is barely one-third of that of the ventricle.

STAGE V.

***

The material for this stage consists of one embryo which measures 3.14mm. in length.

DESCRIPTION OF THE GRAPHIC RECONSTRUCTION OF THE HEART OF A FERRET EMBRYO 3.14mm. IN LENGTH WITH 13 - 14 SOMITES. (F.15d(e)).

The general development of this embryo is so similar to that of the Stage IV specimen as to merit no separate description.

The specimen is slightly older than the Stage IV ferret embryo. As in the case of the other embryos described the state of preservation of this embryo is perfect. In length, it undoubtedly exceeds the Stage IV specimen, but when other measurements are taken, the fact is revealed that the embryo in question is relatively a small one. The pericardial cavity measures 400μ. in its cranio-caudal diameter and 840μ. from side to side.

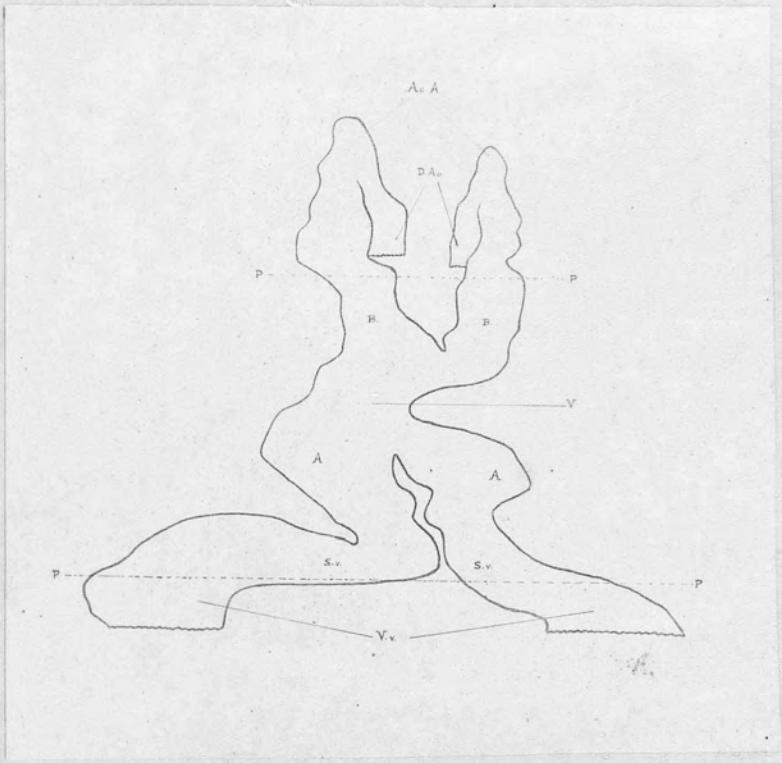
No plastic reconstruction of this embryo has been made. The graphic reconstruction of the heart, however, shows that the two endothelial tubes have united in part of their extent (Figs. 31 and 32).

The fused portion, extending through some

sixteen

FIGURE 31. X 100.

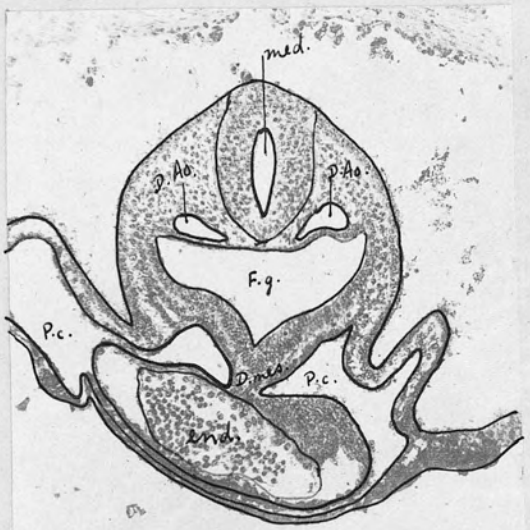
VENTRAL VIEW.



V.v. - Vitelline vein.
S.v. - Sinus venosus.
A. - Atrium.
V. - Ventricle.
B. - Bulbus cordis.
Ao.A - Aortic arch.
D.Ao. - Dorsal aorta.
P. - Reflection of Pericardium.

FIGURE 32. X 100.

SECTION THROUGH FUSED VENTRICULAR REGION.



med. - Medullary tube.
F.g. - Fore-gut.
D.Ao. - Dorsal aorta.
D.mes. - Dorsal Mesocardium.
P.c. - Pericardial cavity.
end. - Fused ventricular portion of the two endothelial tubes.

sixteen sections of 10μ. each, appears to be the ventricular part and lies more to the right side (Figs. 31 and 32). Cranially the fused ventricle divides into two vessels, each of which represents the bulbus cordis (Figs. 31 and 33) and becomes continuous with its corresponding dorsal aorta by looping round the cranial end of the pharynx, thus constituting the first cephalo-aortic arch (Fig. 31). The paired atria run into the fused ventricle cranially and each receives its sinus venosus caudally (Fig. 34).

The vitelline veins are very much in the same stage of development as those observed in Stage IV (Compare figs. 27 and 31). The right vein pursues a more transverse course latero-medially and terminates at its corresponding sinus venosus. In this, as in the preceding specimens, there is no trace of a ventral mesocardium. The dorsal mesocardium is, however, present in this specimen (Figs. 32, 33 and 34).

It may be observed that at this stage of development the ferret heart, though resembling very closely the heart of the Stage V Perameles obesula (19.viii.03) described by Miss Parker, yet differs from it in many respects. In both cases the two endothelial tubes have partly fused. In the ferret

the

the fusion occurs in the ventricular region (Fig. 31.). In the Perameles obesula, according to Miss Parker, the bulbus is the only portion of the heart in which the endothelial tubes have actually fused at this stage.

Asymmetry of the two endothelial tubes has also been noted in Perameles embryos. Miss Parker observes that whilst the left heart tube is practically straight, the right tube shows well-marked curvature. In the ferret it has been found that the two tubes, prior to fusion, appear to have been shifted as a whole towards the right side (Fig.27), and that they remain in this position even after partial fusion has taken place (Fig. 31). Two pairs of aortic arches are found arising from the fused bulbus in the Perameles embryo, but only one pair of vessels can be recognized in the ferret embryo at this stage, and these come from the yet unfused bulbus (Fig. 31). In both instances, the ventricular portion is the most dependent part of the endothelial tubes.

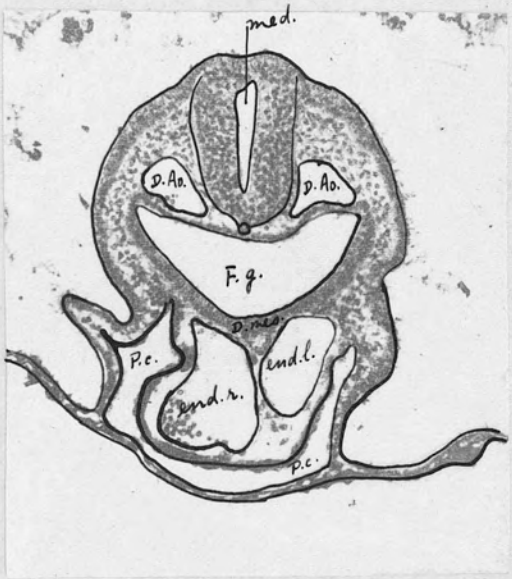
Stage VI Macropus ruficollis 5.2mm. of Miss Parker is distinctly older and is in a more advanced stage of development than the Stage V ferret embryo. In the Macropus the right and left heart tubes are fused except in the region of the sinus venosus,

where

where they remain separate. Three pairs of aortic arches are described. The fused heart has already begun to acquire the S-shaped curvature, so that the bulbus arteriosus lies dorsal to the cephalic extremity of the ventricle. The bulbus arteriosus is continued into a short median ventral aorta which bifurcates to form the first pair of aortic arches. The second and third pairs of aortic arches arise from the median ventral aorta immediately caudal to its bifurcation. The atrial limb of the S. is carried into position dorsal to the ventricle.

FIGURE 33. X 100.

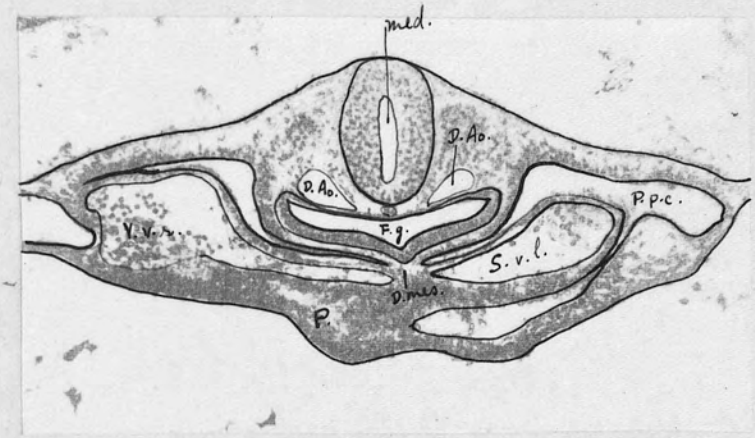
SECTION THROUGH THE BULBUS CORDIS.



med. - Medullary tube.
F.g. - Fore-gut.
D.Ao. - Dorsal aorta.
D.mes. - Dorsal mesocardium.
P.c. - Pericardial cavity.
end.r. - Bulbus cordis of right endothelial tube.
end.l. - Bulbus cordis of left endothelial tube.

FIGURE 34. X 100.

SECTION THROUGH THE SINUS VENOSUS.



med.	-	Medullary tube.
F.g.	-	Fore-gut.
D.Ao.	-	Dorsal aorta.
D.mes.	-	Dorsal mesocardium.
P.	-	Caudal reflection of Pericardium at the level of Septum transversum.
P.p.c.	-	Pleuro-pericardial cavity.
V.v.r.	-	Right Vitelline vein.
S.v.l.	-	Left Sinus venosus.

A VERY EARLY HUMAN OVUM EMBEDDED IN THE UTERUS.

*****

For this interesting human ovum I am indebted to Dr. R. W. Johnstone, Edinburgh University, who has very kindly placed the serial sections at my disposal. The description of the embryonic rudiment of the specimen given by Dr. Johnstone in the Journal of Obstetrics and Gynaecology of the British Empire, May, 1914. , may be quoted in length.

"Owing to the damage received by the specimen the embryonic rudiments are considerably folded and at one or two points broken. It is therefore impossible to identify them with absolute certainty, and I shall in the first place merely describe what is to be seen.

"As one follows through the sections one comes first upon a vesicle which is somewhat crumpled, but is roughly triangular in shape. This vesicle passes through 35 sections and therefore measures in this direction 0.35mm. Its other maximum diameters are 0.39mm and 0.21mm. In most parts it presents two definite layers of cells. The outer layer, which is sometimes very much thinned, contains cells with

oval

oval nuclei. In the thinned parts these are flattened out and arranged parallel to the surface. This layer corresponds in every way to the mesodermic lining of the chorion, and with this tissue it is at one angle of the triangle definitely and substantially continuous. The inner layer is composed of rather larger cells which stain more deeply, and contain large round or oval nuclei with well marked, deeply staining chromatin network.

"The shortest side of this triangular vesicle presents an appearance very like an embryonic area - that is to say, it is composed of cells heaped together in three or more irregular rows. In this area, which measures 0.21 by 0.2mm., the nuclei are more oval, and tend to be arranged perpendicularly to the edge of the vesicle.

"At one end of this 'area' a considerable fold or pocket is given off, which, but for serial sectioning, might easily have been mistaken for a second smaller vesicle. It is, however, directly continuous with the larger portion of the vesicle.

"On the wall of the larger portion there are one or two thickenings which resemble the anlagen of blood vessels. But on tracing these through several sections they are found to be merely localised thickenings, which it is impossible to denote definitely as the precursors of vessels. This vesicle is

unfortunately

unfortunately ruptured at one end, and there are a few maternal red blood corpuscles present within it, as well as some of the mesodermic magma.

"In the later sections containing this first vesicle a second vesicle makes its appearance to the left of the first. This passes through 26 sections and accordingly measures in this direction 0.26mm. Its other maximum diameters measure 0.28mm. and 0.14mm. It is also somewhat crumpled, and is roughly triangular in shape. In structure it is a replica of the first, presenting the same two layers of cells. At the angle farthest away from the first vesicle it also is definitely and substantially attached to the blastocyst wall, its outer layer being for some distance continuous with the mesodermal lining of the chorion.

"In a number of the sections about one-third of the way through this vesicle, the cavity of the vesicle is almost wholly filled up with cells. This flooring of cells appears to divide it more or less completely into two smaller vesicles. This vesicle, like the first, is not absolutely intact, and several red blood corpuscles may be seen in its interior. At no point do the two vesicles come into actual contact, and their attachments to the wall of the blastocyst are towards the opposite extremes of the one side of that structure".

I have examined carefully the whole series of the sections of this ovum and have found that true vascular endothelium in the form of isolated cords is present in the ventral pole of both of the vesicles near their attachments to the blastocysts. Dr Johnstone, however, considers these endothelial cords as merely localised thickenings which he was unable to denote definitely as the precursors of vessels. The reason for the assertion that these cell cords represent true endothelium and not as merely localised thickenings will be found later in the discussion of this communication.

DISCUSSION.

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ORIGIN OF BLOOD CELLS AND VESSELS.

The subsequent development of the blood cells into red and white corpuscles lies outside the scope of the present communication. It is therefore proposed to limit the discussion to the developmental relationships which the blood cells have in common with the vascular endothelium.

In the paragraph dealing with the development of the extra-embryonic vascular rudiments in mammals, it has been pointed out that in embryos of the higher vertebrates, the earliest vascular rudiments have invariably been described by most authors as appearing, at first, in the form of localized cell cords, lying upon the yolk-sac between the mesoderm and the entoderm. His(00) was the first to suggest that these cell cords ultimately develop into blood vessels and blood cells, and the name of Angioblast was, therefore, allotted by this author to these rudiments. Thus far all observations on this point incline to support the work of His. According to the general current of opinion the angioblast is

found

lying between the mesoderm and the entoderm, at first, in the form of cell cords in the area vasculosa immediately surrounding the embryonic shield. It is believed that the peripheral part of the angioblast soon resolves itself into an uninterrupted net-work of endothelium and the central part into clusters of blood cells. It is further stated that the endothelium thus formed is capable of producing new blood cells.

In the literature of the past twenty-five years there are numerous descriptions and illustrations of the origin of blood cells from the vessel linings. In 1892 Schmidt described the transformation of individual endothelial cells into white and red blood corpuscles. In support of this view Maximow (09) states that the endothelial cells and blood cells are closely related and arise from a common stem cell in the blood islands and may continue to do so from such a cell during later development. This author further claims that the intra-vascular primitive blood cells are not only increased by mitosis but are also added to by the proliferation of the same kind of cells from the fixed endothelial wall of the primitive vessels. Minot(12), however, disagrees with Maximow on the ground that there is no protoplasmic continuity of the endothelial cells

with

with the blood cells, and that mitosis of the endothelium in the neighbourhood of the cell clusters is wanting. Minot regards the cells forming the clusters as solely primary wandering cells.

The most damaging evidence against Maximow's view is to be found in the recent work of Stockard (15), who, after having conducted a series of experiments on Fundulus, comes to the conclusion that endothelial lining of vessels is utterly incapable of giving rise to any form of blood cells, and that vascular endothelium arises in loco in many parts of the embryonic body in which blood cell rudiments are not present.

It is obvious that there has not yet been found a solution of whether vascular endothelium and blood cells are interchangeable, that is to say, whether blood cells are capable of metamorphosis into endothelial linings, and whether the latter, once formed, has the power of regeneration into any type of blood cells. A further point regarding the vascular rudiments which is by no means less important, is the genetical origin of the angioblast (blood cell and vascular rudiments). Whether angioblastic cells should be regarded as a derivative from the mesoderm or from the entoderm remains still the subject of much controversy and speculation.

The

The difficulty which has hitherto been encountered in determining the precise mode of origin of these vascular rudiments lies in the fact that nearly all investigators in this field of work have assumed that blood cells and vessels have a common origin. The common rudiment for these two structures is generally spoken of as the 'Angioblast'. This seems to be the chief cause of the great diversity of the results so far obtained, and accounts for the establishment of the two views regarding vascular origin, namely, the mesodermic and the entodermic. In defence of the former, as has already been indicated, the names of Maximow(09), Weidenreich(10), Rückert and Mollier(06) and Stockard(15) are associated, and in favour of the latter, Kölliker(82) and Robinson(92) are of prominence. It appears, therefore, that as long as the blood vessels and blood cells are to be regarded as having a common origin, so long will controversies continue to thrive.

With this in mind, it is the endeavour of the present author to bring forward a few facts which have been observed in the course of investigation into the early stages of development of the main blood vessels and of the heart in the ferret embryo, and, with the aid of these observations, to seek a

clue

clue to the solution of the much contested problem of the origin of blood vessels and blood cells in mammals.

It is proposed that the term 'Angioblast', if used at all, should be limited to endothelial rudiment only. The reason for restricting this term will be explained later.

Against the view that vascular endothelium is capable of producing blood cells, it has been urged that no real vascular endothelial cell has been actually observed to metamorphose into a blood cell or to divide off another cell which forms a blood cell, and until such a direct observation is forthcoming one can only question the accuracy of the interpretation of the various observations up to now recorded. This same argument may be put in the opposite direction, that is, that no blood cell has been credited with indisputable evidence as being capable of being transformed into an endothelial cell.

In the ferret embryos, Stage Ia. and Stage IIb. (Figs. 1. 15b. and 17a.&b.) it is possible to identify mitotic division in the entodermal cells in the neighbourhood of blood cell clusters. There is no evidence to show that in the ferret, endothelial cells are capable of giving rise to blood cells. In figures 2a.b., 3, 4a.b., 5, and 15a., it is to

be

be observed that blood cells are in abundance on the yolk-sac. These are frequently adherent to the entodermal cells which, if not in direct protoplasmic continuity with the blood cells, are, in many cases, in close contact with them. It is to be further noted that where the apposition of these cells is intimate, it is impossible to distinguish the blood cells from many of the entodermal cells, the nuclei, size and shape of the two kinds of cells having a close resemblance. On the other hand, a great dissimilarity exists between the blood cells and the neighbouring mesodermal cells, for in the former, the cells are, without exception, spheroidal in shape, their nuclei are large, staining more deeply and the protoplasm is comparatively small in amount, whilst in the latter, the cells are usually spindle shaped, their nuclei have mostly differentiated and taken on a lighter stain (Figs. 3, 4a.& b., 5, and 15a). Furthermore, in the ferret embryos blood cells have been observed in regions of the yolk-sac where invasion of the mesoderm has not yet taken place. The above facts appears to indicate that, in the ferret at least, if not in all the other mammals, the origin of blood cells from the entoderm is highly probable. For this reason the plea is put forward

that

that the term 'Angioblast' should not be applied to the blood cell rudiment.

With regard to the genetic origin of the vascular endothelium, numerous investigators have recorded wandering mesenchymal cells upon the yolk-sac. Stockard(15) claims that in Fundulus these wandering mesenchymal cells ultimately give rise to four different kinds of cells - the endothelial cells, the black chromatophores, the brown chromatophores and the blood cells.

Another current view is that after the so-called 'Angioblast' has made its appearance, the vascular endothelium arises from the cells of the blood islands by a re-arrangement of the peripheral cells of the blood islands to form lining endothelium and the central ones to remain as blood cells, and that further extension of the endothelium is brought about by buddings of the endothelium which appear, at first, as solid cords but later become hollow. There is so far no convincing evidence to show that the peripheral cells of the blood islands in the ferret are capable of being transformed into endothelial cells. It is to be noted that in the ferret, endothelial cells, whether in the form of solid cords or grouped together with a lumen, are invariably spindle shaped from the very beginning.

Ziegler(87)

Ziegler(87) maintains that the system of blood vessels and that of the lymphatic vessels are produced from the remnants of the blastocoel which remain behind as vessels, lacunae, or interstices. Felix(97), however, inclines to the belief that the circulatory system is, from a developmental point of view, closely related with the coelom.

In connection with this question Stockard(15) states: "The vessels arising from independent mesenchymal cells in the space of the blastocoel in the teleost yolk-sac entirely overthrow any notion that vessels arise ontogenetically as portions of the coelomic epithelium. The vascular lumen is originally continuous with the primary body cavity, the segmentation cavity, and never with the secondary body cavity or coelomic cavity."

It is clear that these authors agree, at least, that the origin of the endothelium is from the mesoderm. In the ferret it is possible to demonstrate that endothelial cells take their origin from the splanchnic layer of the mesoderm. In some of the sections of the ferret embryo of Stage IIa. (Figs. 10a - d) there are indications to support the view of Felix(97) that portions of the coelomic space surrounded by mesoderm may be cut off to form vascular endothelium and to lie between the mesoderm and entoderm.

The

The opinion that endothelium develops independently of blood cells is further strengthened by the observations of Stockard(15), who finds that vascular endothelium arises in loco in many parts of the embryonic body of the Fundulus, in which blood cell rudiments are not present, and that independent blood islands, having no connection with the intermediate cell mass, are found on the yolk-sac, and even in extremely young embryos blood islands may appear on the ventral yolk surface at a great distance away from the intermediate cell mass. He maintains also that early blood islands are invariably destitute of endothelial linings. Though favouring the view that vascular endothelium and blood cells are independent of each other in their mode of development, Stockard (15) firmly believes that both types of cells are derived from wandering mesenchymal cells. His recent conclusions on the development of the vascular endothelium and blood cells, which appear in the November issue of the Amer. Jour. of Anatomy vol.18. No.3. 1915., may, for the sake of comparison, be summarised as follows:

1. The mesodermal layer in Fundulus embryos is represented by numerous separate wandering mesenchymal cells. These cells migrate away chiefly from the caudal end of the

embryo

embryo between the ectoderm and the entoderm (periblast of the yolk-sac). When the cells first make their appearance they are all closely similar in shape and size.

2, Later the wandering mesenchymal cells begin to differentiate into:

a, Elongated spindle cells with delicate filamentous processes (endothelial cells). These cells tend to group themselves into more or less irregular collections. "This cellular aggregation may then be regarded as the actual anlage of the vascular endothelium of the future vessel. The anlage consists merely of a group of separate wandering mesenchymal cells and not of a capillary net in any sense". The endothelial cells never metamorphose into blood cells.

b, Amaeboid cells with conical pseudopod-like processes (Black and Brown chromatophores).

c, Small circular cells with thick pseudopods (blood cells). These cells wander out later than a. and b. and from a limited region - the caudal end of the embryo. They tend to

cover

> cover the caudal surface of the yolk-sac and to reach its ventral aspect. Groupings of the blood cells next take place on the caudal and ventral yolk surfaces where they constitute early blood islands. At first the blood islands are not surrounded by endothelial cells but later become enclosed or taken into the ends of the incipient vessels.

Stockard summarises his statement by saying: "The differences among the four types (endothelial cells, black chromatophores, brown chromatophores and blood cells) produced are from the stand-point of our present knowledge in all probability due to the potential differences among the apparently similar mesenchymal cells from which they arose. The four types including endothelial cells and erythrocytes we must consider from an embryological stand-point as arising from different mesenchymal anlagen".

It will be observed then that Stockard, whilst admitting, on the one hand, that (a) endothelial cells are quite different from blood cells in shape, in position, and in the period of migration; that (b) the former develop independently of the latter; and that (c) blood cells when first formed are devoid of

endothelium

endothelium, claims, on the other hand, that both types of cells have a common parent trunk - the wandering mesenchymal cells.

In the Fundulus this is perhaps true, but it is erroneous to presume that in mammals a similar phenomenon occurs.

In the ferret there are indications to suggest that vascular endothelium is mesodermic in origin and that blood cells may, at least, be added to, if not derive solely, by the proliferation of the entodermal cells. Such being the case, it is obvious that the sources of origin of the blood cells and vascular endothelium are distinct, and that these two different vascular rudiments cannot be considered to have a monophyletic origin.

If the biphyletic origin of blood cells and vascular endothelium is to be accepted, one more point still remains to be solved, namely, how, when, and where the first blood cells enter the circulation. This has been variously described not only in embryos of different species but probably even among embryos of the same species. Ziegler thinks, however, that just beyond the lateral plates in the plasma filled spaces of the yolk-sac which lie between the periblast and ectoderm, the first blood cells project into the circulation. This statement has unfortunately not been substantiated by sufficient evidence and cannot, therefore, be accepted as conclusive. Stockard

describes

describes that in Fundulus embryos the earliest blood cell formation occurs in the yolk-sac blood islands. The cells in these islands continue to divide until they become surrounded by endothelium. Precisely how these blood cells are provided with endothelial covering, Stockard makes the following statement: "A growing vascular tip may be observed at certain stages to come in contact with a group of erythroblasts, or actually a blood island unsurrounded by vascular endothelium. The tip of the vessel seems to disorganize to some extent and its cellular elements slowly surround the group of corpuscles which are later taken into the circulation as the current becomes established in the including vessel".

In ferret embryos it is difficult to determine the exact moment and the precise manner when blood cells get into the vascular endothelium, but there is evidence to indicate, however, that the earliest blood cells are not surrounded by endothelium, and that later they become engulfed by spindle cells which grow round them to form vascular endothelium (Fig. 16). These spindle cells have in most instances been traced to the splanchnic layer of the mesoderm.

INTRA-EMBRYONIC BLOOD VESSELS.

In the review of the literature on the subject of the origin of the intra-embryonic blood vessels, it has already been indicated that the problem is one of the most difficult to be solved in the embryology of the vertebrate animals. Numerous conflicting views have been advanced regarding the precise mode of the origin of the intra-embryonic blood vessels. Thus His(00) and Hertwig(92) have associated themselves with the theory that the early blood vessels in the body of the embryo are formed by a budding or ingrowth of the endothelial lining of the vessels from the extra-embryonic vascular area, and Sobotta(02) supports the belief that vessels in the embryo develop in situ, and those on the wall of the yolk-sac are secondary as a result of an outgrowth from the intra-embryonic blood vessels. Rabl(86), on the other hand, pointed out the possibility of the vessels of, at least, the cranial region, if not the whole vascular system of the embryo, having been formed by the extension of the paired heart rudiments when these are developed. Recently Rückert and Mollier(06) maintain that the embryonic vascular system, or at least a part of it, arises in situ from the mesoderm of the embryo. Rückert(88) claims

that

that in Selachians, the aorta develops in loco. Felix(97) states that in birds, the aorta and certain venenplexus all arise in loco.

Each rival view therefore is still open for further critisms and invites additional support. In birds the caudal portion of the dorsal aorta is, according to Vialleton(92), His(00) and Evans(09), formed from the medial margin of the vitelline plexus which has grown into the embryo in the manner already indicated in the beginning of this communication. Türstig(84) also has noticed the frequent early connection of the primitive dorsal aorta with the vitelline plexus in mammals. On the other hand, Sobotta(02) imagines that the dorsal aorta is the primary vessel in the embryo, and that vascular sprouts spring from it to form other vessels. In the ferret, Stage IIa. 1.97mm., it has been found that the caudal portion of the dorsal aorta has established its communication with the vitelline plexus. Precisely how the caudal end of the dorsal aorta in the ferret is developed, no definite statement can be made, but as far as evidence goes, it is probable that this part of the dorsal aorta arises much in the same way as described by Vialleton(92) and His(00).

For the development of the cranial portion of the

the dorsal aorta, on the other hand, various opposviews are held. His(00) attributes it to the result of a further growth of the same extra-embryonic vitelline plexus which forms the caudal part of the aorta, but which is reduced to a capillary chain growing cranially, eventually turning ventrally over the blind end of the fore-gut and fusing with the cranial portion of the heart tubes. In support of this theory Lewis(04) affirms that all intra-embryonic blood vessels of rabbits are apparently derived as off-shoots from the extra-embryonic net-work of vessels in the splanchnopleure of the yolk-sac, the vitelline plexus ending medially in the embryo in the form of two vessels - the dorsal aortae. Quite recently Bremer(12) states that in the rabbit embryo of 5 somites, the dorsal aorta, the first aortic arch, the conus arteriosus and the lateral heart are all parts of an original net-work of angioblastic cords derived from the extra-embryonic plexus of blood vessels.

Rückert and Mollier(06) maintain that the cranial portion of the aorta is developed in situ from the mesodermic cells of the lateral plate of the mesoderm of the cranial region of the embryo. Mollier(06) says: "the notion of His(75) and Viallеton(92) that the vessel strands of the embryo grow

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in as sprouts from the extra-embryonal rudiment is not nearly so probable as that the individual vessel cells arise in loco and thus form the vascular net". In support of the autochthyonic origin of the cranial portion of the dorsal aorta, the work of Huntington(10, 14) and McClure(10, 12) may be cited. Recently this view is further strengthened by the results of the experiments of Miller and McWhorter (14) on the origin of blood vessels in the chick embryo. Further support is to be found in the more recent experimental evidence, presented by Reagen (15), which shows the origin in loco of vessels in isolated parts of chick embryos, and by Stockard(15), which claims beyond doubt that in Fundulus embryos the heart endothelium and aorta arise in loco within the embryo, and here there are no vessels, nor even mesoderm, present on the yolk-sac in the cranial portion.

Figure 6a. represents the graphic reconstruction of the vascular system of the cranial portion of the Stage IIa. ferret embryo. In this specimen the heart rudiment is represented merely by a transverse blood channel which lies across the median plane and unites the cranial ends of the two vitelline veins. The pleuro-pericardial cavity together with the pleuro-pericardial canals has already been

described

described as having the shape of an inverted U-shaped canal which lies dorsal to the vitelline vein and the heart rudiment. Two rudimentary dorsal aortae can be made out in this specimen. They run caudo-cranially one on each side of the medullary groove. They are still more or less plexiform in character, and they terminate blindly at their cranial extremities. The absence of the first aortic arch, which is so conspicuously seen in the next stage, deserves particular notice. It is clear that at this stage of development in the ferret, the heart rudiment and the caudal part of the dorsal aorta are present, but the connection, i. e. the cranial dorsal aorta, the first aortic arch and the conus arteriosus, between the heart and the caudal dorsal aorta, is still wanting (Fig. 6a). A stage further in the development of the cranial portion of the dorsal aorta is illustrated by the Stage III embryo (Fig. 18). Here the dorsal aorta is seen to have established its connection with the heart rudiment through the first aortic arch. But exactly how this connection takes place, there is no evidence from which to form any definite conclusion. It is as yet impossible to decide whether the first aortic arch and the conus arteriosus when developed, as seen in the specimen just referred to, should be attributed to the result

of

of a cranialward growth from the dorsal aorta, or as the direct outcome of an extension of the heart rudiment growing round the cranial end of the fore-gut to join the dorsal aorta and to constitute the conus arteriosus and the first aortic arch. All that can be said is that probably coinciding with the formation of the head-fold the two dorsal aortae are carried, pari passu, cranialward over the cranial end of the fore-gut, and possibly, as the result of a further growth from the blind ends of the aortae towards the heart rudiment, these structures establish their communications with the heart. If this contention represents precisely what really takes place in the ferret embryo, the conus arteriosus and the first aortic arch must be considered as being the result of a further growth from the dorsal aorta. But if the other theory is to be accepted, that is that the development of the conus arteriosus and the first arch is due to an extension of the heart growing round the fore-gut, then the development of these parts of the vascular system conforms with the statement made by Bremer(12) relating to the early development of the blood vessels in the rabbit embryo of 5 somites. This investigator asserts that the dorsal aorta, the first aortic arch, the conus arteriosus and the lateral heart are all parts of an original

net-work

net-work of angioblast cords derived from the extra-embryonic plexus of blood vessels. Another mode of origin which cannot, however, be overlooked, is that, after the heart rudiment and the dorsal aorta have been laid down, the remaining parts of the main vascular system of the cranial region of the embryo may develop in situ from the mesoderm. The view that parts of the intra-embryonic vascular system arise in situ cannot be ignored, for there is an overwhelming accumulation of conclusive evidence to indicate that the formation of intra-embryonic blood vessels is much more extensive and important than has hitherto been supposed.

From whichever point of view the development of the dorsal aorta, the first aortic arch, the conus arteriosus and the heart is to be looked upon, the fact remains that these structures do not develop simultaneously. This is clearly shown in Figure 1a, in which the heart rudiment and the dorsal aorta at this stage of development are all represented, yet there is nothing to indicate or to represent the future first aortic arch and the conus arteriosus. With these facts in hand one is justified in saying that the development of the first aortic arch and the conus arteriosus, though closely related, on the one hand, to each other and the heart, and, on the other hand, to that of the dorsal aorta, is really secondary and occurs after the heart rudiment and

the

the dorsal aortae have made their appearance.

DEVELOPMENT OF THE HUMAN VASCULAR SYSTEM

In man, on account of the comparatively small size of the yolk-sac, the development of the earliest blood vessels undergoes some important modifications. In birds and reptiles, it should be recollected that the chief source of nutrition for the growing embryo is solely from the yolk-sac, which is of great dimensions. In birds and reptiles then, it is obvious that the circulation of the embryo, once established, should be connected with the yolk-sac. This is spoken of as the early vitelline circulation. The allantois in birds and reptiles, though persistent during incubation, is not important as a nutritive organ. In man the allantoic diverticulum from the gut is rudimentary but the allantoic mesoderm plays an important part in the formation of the placenta which establishes the connection between the embryo and the mother, and its vessels which correspond to the allantoic vessels in reptiles and birds become associated with the placental circulation.

The essential differences in the development of the human vascular system having been thus broadly indicated, a more detailed account of the subject, as far as it is known to-day, may now be entered upon. According to Evans(12), it is certain that

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in man, long before any vascular rudiments are found in the body of the embryo, and at a time before any mesodermic somites are formed, typical vascular rudiments are detected irregularly scattered, at first, over the surface of the ventral pole of the yolk-sac only, but on account of its comparatively small size the vascularisation of the whole surface of the yolk-sac is soon completed. Minot(12), however, thinks that in man the vascular area covers the whole surface from the beginning.

It is generally believed that, as in other vertebrates already studied, these vascular rudiments make their appearance as nodular swellings of that part of the wall of the yolk-sac known as the area vasculosa, and are cell clumps lying between the mesoderm and the entoderm. It is claimed also that very shortly after their appearanec, the peripheral cells of these cell clumps arrange themselves to form endothelium while the central ones remain as blood cells.

In young human embryo it has been possible to demonstrate that, at a period before any vascular rudiments on the yolk-sac proper can be distinguished, there develop in the belly-stalk and chorion of the embryo indisputable blood vessels which appear, at first, as strands of spindle cells possessing a lumen.

This

This has been described by Fetzer(10) and also observed by Graf Spee(96) in the embryo Von Herff of .37mm. Others (Jung(07) and Herzog(09) have called attention to the aggregations of endothelial cells in the belly-stalk. True blood islands in the belly-stalk near the allantois have been described also by Grosser(13) and Debeyre(12). Frassi(08) also is in favour of the view that well-formed angioblastic cords can be detected on the ventral surface of the yolk-sac and in the belly-stalk and chorion.

Judging from the observations made by these investigators, it is feasible to believe that what has been considered as the "mere localized thickenings" described by Dr Johnstone in his first human ovum, is, in reality, true vascular endothelium. The contention for this belief is based on the fact that, in positions and in their general characters, these cell cords or 'thickenings' bear a close resemblance to those of other early human embryos in which similar cells have been described as endothelium by Fetzer(10), Graf Spee(96), Jung(07), Frassi(08) and others.

Recently Bremer(14) pointed out that, in human embryos, the earliest blood vessels appear separately in the yolk-sac and in the belly-stalk in the form

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of multiple rudiments which are for the greater part funnel-shaped invaginations of the surface of the mesoderm. By a partial fusion of the walls of an ingrowth, a portion of the coelom, bordered by mesoderm, may be cut off as a separate cavity, lying deep within the substance of the belly-stalk. This investigator, therefore, believes that the endothelium arises either by delamination from the walls of such a detached portion of the coelom, or by direct extension, in the form of an angioblastic cord, from the mesothelial ingrowth.

Most authors believe that the early development of the vascular rudiments in the belly-stalk and chorion in human embryos happens before the yolk-sac proper exhibits any vascular elements. That this should be the case, as already pointed out, is due to the fact that in human embryo the vitelline circulation is of secondary importance. The belly-stalk and chorion, on the other hand, constitute the primary connection between the embryo and the placenta and are therefore the first to be vascularized. This deviation from the ordinary type of development is but one of the remarkable series of variations with which man is distinguished from his fellow creatures.

After the vascular rudiments have made their appearance in the belly-stalk and chorion, the yolk-sac proper is next vascularized. This is clearly

seen

seen in the human embryo 1.17mm. described by Frassi (08) in which there is an abundance of well-formed vascular elements on the ventral surface of the yolk-sac, and with very little or no difficulty, vessels can be detected also in the belly-stalk and chorion. As already indicated in the review of the literature relating to the development of the human vascular system, the first intra-embryonic vascular rudiments appear after the belly-stalk, chorion and the yolk-sac proper are all vascularized. This stage is illuatrated by the well-known embryo Glaevecke 1.54 mm. of Graf Spee(89, 96). Here, as in the preceding stage, vascular rudiments are seen on the yolk-sac and in the chorion, but in addition to these, it is possible to note the first intra-embryonic vascular rudiments. In the region of the heart these intra-embryonic vascular rudiments exhibit the characteristic appearance of endothelial cells, and in the more caudal portion of the embryo, strands of clearly isolated and differentiated cells are found lying between the entoderm and the mesoderm; they therefore represent nothing else but the intra-embryonic vascular cells (angioblast). Finally, as the allantoic mesoderm grows, they can be followed into vessels which, in the belly-stalk, lie at first on each side and soon below the allantoic diverticulum. The vascular cells are, without doubt, to be looked upon

as

as the rudiments of the umbilical arteries in the belly-stalk.

The fact seems to be established that, in man, the first vascular endothelium is laid down in the belly-stalk and chorion. According to Bremer(14) it seems clear that in human embryos, the earliest blood vessels appear separately in the yolk-sac and in the belly-stalk in the form of multiple rudiments. The rudiment in the belly-stalk and probably also in the yolk-sac are funnel-shaped ingrowths of the surface mesoderm. By a partial fusion of the walls of an ingrowth, a portion of the coelom, bordered by mesoderm, may be cut off as a separate cavity lying deep within the substance of the belly-stalk. Bremer claims that the endothelium arises either by delamination from the walls of such a detached portion of the coelom, or by direct extension, in the form of an angioblastic cord from the mesothelial ingrowth, and that future extension is by means of the angioblast cords, which grow apparently through the surrounding mesoderm. No blood cells have been described to have developed in the belly-stalk and chorion along with the endothelium. It is obvious that the angioblast in the belly-stalk and chorion gives rise to endothelium only, and that they are mesodermic in origin. On account of this difference of origin it has been proposed to limit the term 'angioblast' to

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the endothelial rudiment only.

This furnishes an additional evidence in favour of the biphyletic origin of blood cells and blood vessels. The next question to be considered is whether the vascularization of the yolk-sac proper is to be regarded as the extension of a further growth from the vascular rudiments in the belly-stalk and chorion, or whether the process arises in situ by separate vascular rudiments. Bremer(14) thinks that the vascularization of the yolk-sac proper is a separate manifestation, but it is quite possible that the first vascular endothelium of the yolk-sac is the result of an extension from the endothelium of the belly-stalk and chorion.

Regarding the intra-embryonic blood vessels there are evidences to show that the vascular endothelium in the cranial region of the human embryo arises in situ and that in the caudal region this is probably due to a spreading of the extra-embryonic endothelium into the caudal end of the embryo.

DEVELOPMENT OF THE HEART AND PERICARDIUM.

In the literature dealing with the development of the heart and pericardium, much has been written regarding the developmental processes of these structures in mammals, but in man much is required yet before a comprehensive knowledge of the developmental phenomena can be obtained. It is still a speculation if the earliest rudiment of the human heart is essentially similar to that of the mammalia. Tandler(12) inclines to the belief that they are similar. It is not to be expected, at present, that the comparison of the development of the human heart with that of the mammalia will throw much light upon the subject until the various developmental processes of the different members of the mammalian embryos are first understood.

In mammals, as has been stated previously, the first rudiment of the heart is the appearance of a number of cells - the angioblast of His, which are distinguishable in embryos of 2 - 3 primitive somites. These vascular cells appear between the entoderm and mesoderm in the cranial region of the embryo on each side not far from the median plane. They are responsible for the development of the endothelial heart tubes only, the remaining structures of the wall of the heart, that is, the myocardium

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and the epicardium, being derived from that part of the visceral coelomic wall which has been designated by Mollier(06) the heart-plate or cardiogenic-plate. It is generally believed that the first appearance of the vascular cells of the mammalian heart is bilateral and is located on the ventral aspect of the pleuro-pericardial canals. Spaces soon make their appearance in the vascular cell masses, and when these coalesce, two endothelial heart tubes are formed, one on each side of the median plane of the embryo. Hensen(75) is credited as being the first who observed this bilateral origin of the heart. A fusion of the two endothelial tubes next occurs and the paired heart rudiments are therefore transformed into an unpaired heart tube, but the precise mode of this transformation is, up to the present, sub judice.

As far as the bilateral origin of the heart rudiments is concerned there seems to be still a little doubt. In all vertebrates that have been investigated all writers incline to believe that the heart rudiments originate in two lateral parts, but precisely how these two parts are brought together to form an unpaired heart is much a disputed point.

As already pointed out (vide supra), some believe (a) that in mammals, as in birds, the two endothelial tubes, out of which the heart is formed, appear at a time when the lateral folds which are

said

said to form the ventral wall of the throat are only just visible; (b) that, on the formation of the lateral folds of the splanchnic walls, the two halves of the heart, enclosed within the hitherto symmetrical and laterally placed pleuro-pericardial cavities, become carried medially and ventrally until they fuse on the ventral aspect of the fore-gut; and (c) that the heart is therefore provided, at least for a time, with a ventral and a dorsal mesocardium.

Balfour(81), as will be remembered, states: "the heart should only be formed as two tubes when it arises prior to the formation of the throat"(mammals), and "as a single tube when formed after the formation of the throat"(elasmobranchii, amphibians). He concludes by saying: "the formation of the heart as two cavities is a secondary mode of development, which has been brought about by variations in the period of the closing in of the wall of the throat".

Professor Wilson of Sydney(14), writing in favour of the presence of a ventral mesocardium in human embryos, asserts that the human heart, like the amphibian, has, at a certain period of development, a ventral mesocardium. This author bases his conclusion on the result of the examination of a series of sections of a human embryo measuring 1.78 mm. catalogued H3 in his series, which was aborted

and

and is admittedly in an indifferent if not bad state of preservation. In addition it is stated that the specimen was cut 'in a plane intended to be transverse to the long axis of the embryo, but which turned out to be distinctly oblique', The obliquity appears to have taken place in two planes instead of one, namely, a ventro-dorsal and a lateral. No reconstruction of the heart of the embryo has been made to verify his statement and the number of somites has been hesitatingly determined to be three pairs.

In figure vi of Wilson's paper, an incomplete 'septum' in the pericardial cavity has been named by him the 'septum proprium interpericardiacum'. This he looks upon as evidence of the bilateral origin of the pericardial cavity, which, according to his observation, ends on each side 'blindly without establishing any communication with other coelomic cavity such as occurs later, e.g. in Mall's embryo, No. 391'. In the same figure the heart appears to have an attachment to the ventral wall of the pericardium which presumably Wilson calls the ventral mesocardium.

It is quite possible, as far as the figure shows, that the so-called septum proprium interpericardiacum is merely a fold of the pericardial wall which has never, at any time, formed a true septum.

The

The statement made regarding the blind termination of the pericardial cavity on both sides of the embryo is contrary to all known facts established by other observers on this structure in mammals. The narrow communication between the pericardial cavity and the coelomic cavity has probably been overlooked by Wilson in his specimen on account of the poor histological details of the sections, or the pleuro-pericardial canal might have been obliterated in such a way as to render the communication unrecognizable. If the observation of Wilson is the true interpretation of the condition of the pericardium at this stage of development, then the balance of evidence in favour of the primary communication of the pericardial cavity with the general coelomic cavity in mammals is totally upset. But the unsatisfactory condition of the sections, from a histological point of view, unfortunately affords ground to doubt the accuracy of the observation, otherwise this embryo opens a new field for further investigations into the true nature of the pericardial cavity at this particular period of development in the human embryo. The connection between the heart tube and the ventral wall of the pericardial cavity in Wilson's specimen can be feasibly explained in the following way. If the obliquity of the plane of section is such that

it

it passes through the septum transversum as well as the heart tube situated immediately cranial to it, it will be seen that what appears to be the ventral mesocardium is really a part of the septum transversum. Moreover, it is quite possible that what Wilson considers to be the two endothelial tubes, may after all, turn out to be the two vitelline veins passing through the septum transversum to reach the sinus venosus cranially, and that the specimen he has been dealing with might have been pathological.

I was fortunate enough to have at my disposal also the second young human embryo which has been described by Dr Johnstone(14) in his "Contribution to the study of the early human ovum". When the sections came to my hand, it was found that the series is unfortunately not entirely complete; consequently a reconstruction of the heart of this interesting stage had to be abandoned. Nevertheless the series serves one particular purpose very well, namely, that it shows that this specimen has been cut in a plane not dissimilar to that of Wilson's embryo, and that the heart tube in Dr Johnstone's specimen bears a close resemblance to that of Professor Wilson's specimen, in so far as the anatomical relationships of the heart are concerned.

Though no description of the heart of the

embryo

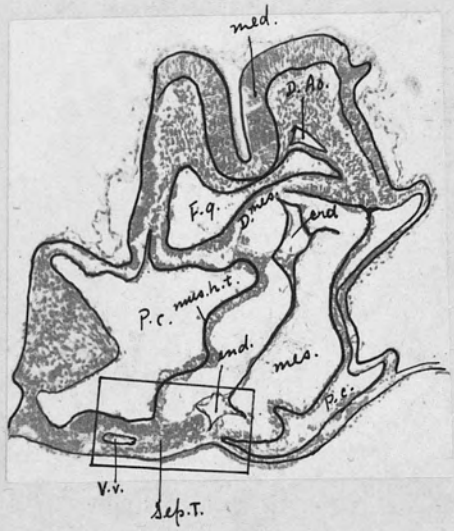
embryo has been made by Dr Johnstone in his paper, a few very interesting points can be detected by an examination of the serial sections. At first glance the section of Dr Johnstone's specimen, from which figure 35 is taken, appears to indicate that the heart has a ventral mesocardium connecting the ventral part of the heart tube with the ventral wall of the pericardium. But on closer examination, what seems to be ventral mesocardium is found to be really part of the septum transversum, for when this structure is traced forwards and backwards in the series it is found that the vitelline veins traverse it to join the sinus venosus. Figure 36 X 500 shows that the vitelline vein passes through the septum transversum to reach the heart. Cranial to this there is nothing to indicate a ventral mesocardium, the cavity of the pericardium extending without interruption from side to side (Fig. 37). Professor Robinson has very kindly looked through the sections of Dr Johnstone's specimen with me and has confirmed the above statement.

In the chick, on the other hand, a ventral mesocardium is recognisable, but this is due, as Robinson(02) points out, to the relatively late penetration of the pleuro-pericardial canals into the mesoderm in the cranial region. The pleuro-

pericardial

FIGURE 35 X 100.

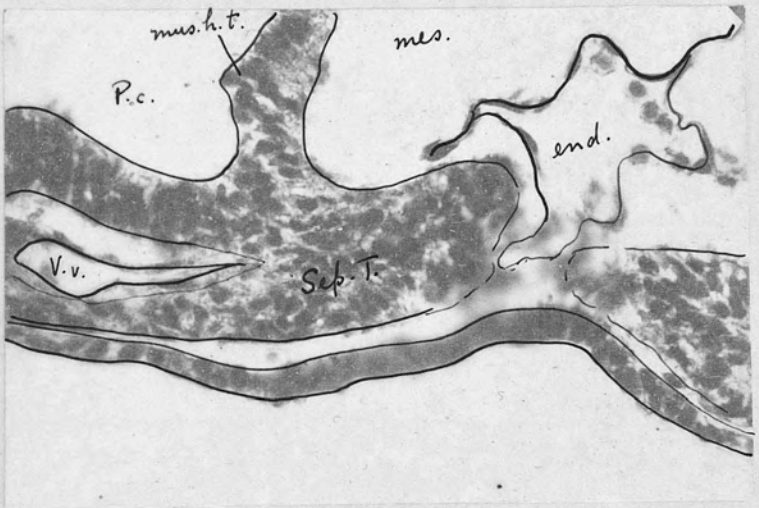
HUMAN EMBRYO.



med. - Medulla.
F.g. - Fore-gut.
D.Ao. - Dorsal aorta.
D.mes. - Dorsal Mesocardium.
P.c. - Pericardial cavity.
end. - Endothelial tube.
Mus.H.T.- Muscular Heart Tube.
mes. - Mesodermic tissue.
Sep.T. - Septum Transversum.
V.v. - Vitelline vein.

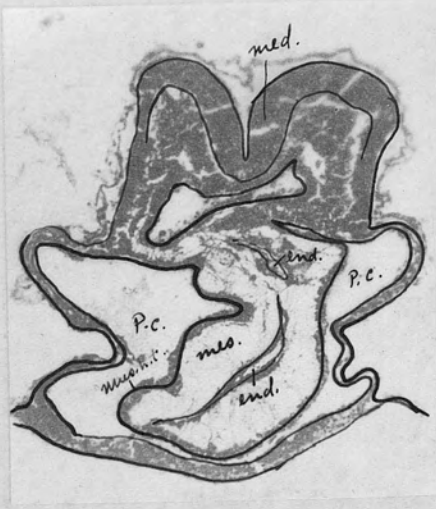
FIGURE 36 X 500.

(Magnified from the Square in Fig. 35)



Same as in Fig. 35.

FIGURE 37 X 100.



Same as in Fig. 35.

pericardial canals do not extend round and unite in front of the medullary plate in early stages, but only at a later stage do they penetrate into the floor of the fore-gut after the mesoderm has been formed. The lateral cavities therefore do not at once become continuous, but remain separated from each other by a double layer of mesoderm constituting the ventral mesocardium. In amphibians lateral folds have been described, but it is erroneous to presume that such folds which, by virtue of their fusion ventrally, form the ventral wall of the fore-gut, really occur in mammals.

According to Robinson(02) the pericardial mesoderm appears in the pericardial portion of the embryonic area, and it is there completely differentiated into somatic and splanchnic layers before the head bend is developed; there is, therefore, a single pericardial cavity to begin with, which extends from side to side along the cranial boundary of the embryonic area. As the head bend develops, the single pericardial cavity is reversed, and it is carried into the ventral wall of the fore-gut, where it forms a U-shaped tube which communicates at each end with the general coelom. The heart rudiments are formed in the splanchnic layer of the pericardial mesoderm; therefore after the reversal of the area,

they

they lie in the dorsal wall of the pericardial cavity attached only by a dorsal mesocardium to the ventral wall of the fore-gut, but they are never, at any time, connected with the ventral wall of the pericardium by a ventral mesocardium.

Rouvière(04), whilst agreeing with Robinson as to the absence of the ventral mesocardium, propounds a different interpretation of the process which brings about the closure and development of the fore-gut. This author asserts that the lateral pleuro-pericardial canals grow cranially round the cranial end of the brain-plate and fuse across the median plane to form a continuous channel - the pleuro-pericardial cavity. The splanchno-pleure forming the caudal wall of the pleuro-pericardial cavity now forms a continuous fold which Rouvière calls the 'cardiac fold', and he affirms that it grows actively caudal-wards as a whole. In this way the fore-gut is closed ventrally.

Gräper(12) takes up an intermediate position. In his description of the growth processes in the developing chick embryo, which he worked out by staining the living embryos and keeping them under observation while still alive, he shows that there is considerable evidence to prove that the margin of the fore-gut (umbilical orifice) moves caudally concurrently with the growth of the head fold cranially.

Miss Parker(15), in her investigation into the early stages in the development of Marsupials, summarises her statement in support of Rouvière by saying "that while the initiation of head fold formation is in all probability due to the forward growth of the brain-plate, there occurs also an active backward growth of the anterior intestinal portal (umbilical orifice), this process is associated with the rapid expansion of the pericardium which occurs at this period of development, and which brings about the backward and inward growth of the layer of splanchnopleure limiting the pericardium".

Against the view that a backward growth occurs in the cranial margin of the umbilical orifice, is the contention of Robinson(02) that "the orifice (of the umbilicus) is not reduced in size during the early stages of development by the convergence of its margins towards a central point. This being the case, no tucking off of the embryo from the surface of the ovum can occur; on the contrary, what does occur is almost the exact opposite of such a process, for the margin of the area remains as a relatively slow-growing region, whilst the embryonic and extra-embryonic portions of the wall of the ovum rapidly increase in extent. Under these circumstances, it follows that the margin of the embryonic area will soon appear as a ring between the upper or embryonic

and

and the lower or extra-embryonic parts of the ovum, both of which have expanded beyond it in all directions".

Having thus briefly brought forward the various views regarding the development of the pericardium and the first appearance of the heart rudiments, it will be of interest to consider the evidence afforded by the material described above. It is the endeavour of the present author to show that it is entirely in accord with the view that in mammals there is no ventral mesocardium, a fact which, in itself, is a strong argument against the theory that gut closure is effected by the fusion of lateral folds.

In the Stage IIa. & b. in which the head fold of the ferret embryo has not yet appeared (Figs. 6a., b., c.,7a.& b., 11a.& b.), the pleuro-pericardial cavity has grown across the median plane, and immediately caudal and ventral to this,"primary" union of the two halves of the hitherto bilateral heart rudiments has taken place at the median plane. It should be specially noted that the term "primary" proposed by the author signifies a "secondary" union after the heart rudiments have once more returned to the condition of bilateral endothelial tubes. To this point reference will be made subsequently in connection with the discussion of the formation of the unpaired heart rudiment from the two separate endothelial tubes.

At Stage II of development the ferret embryo shows that the pleuro-pericardial cavity and the heart rudiments are all represented before there is any indication of the formation of the fore-gut or the head fold (Fig. 6a). It should also be noticed that when the heart rudiments and the pleuro-pericardial cavity first make their appearance the former invariably lie ventral to the latter (Figs. 6b. and 8). In the subsequent stages of development (Stage III and onwards) a change takes place in their position, with the result that the heart rudiments occupy a plane dorsal to the pleuro-pericardial cavity and are connected with the ventral aspect of the fore-gut only by the reflection of the splanchnic wall of the pleuro-pericardial cavity. The reversion of positions of the heart and the pleuro-pericardial cavity can only be explained, as suggested by Professor Robinson (O2), by a forward growth of the head fold to form the fore-gut with the cranial margin of the umbilical orifice remaining stationary. With the development of the head fold the heart rudiments suffer a rotation round a transverse axis over the pleuro-pericardial cavity. Consequently when the two heart rudiments are brought together to form one endothelial tube, it is connected with the ventral surface of the fore-gut only by a dorsal mesocardium, which is formed by the

reflection

reflection of the splanchnic layer of the pleuro-pericardial cavity over the lateral and ventral surfaces of the two heart tubes; the portion of the splanchnic wall of the pleuro-pericardial cavity lying between their medial surfaces having, in the meantime, been pushed ventrally. In this way there is no fusion of this part of the pleuro-pericardial wall, nor does any fusion occur ventral to the heart tubes to form the ventral mesocardium.

Since the time of Hensen(76), the first appearance of the heart rudiments has been considered to be bilateral, that is to say, the heart rudiments develop as two separate endothelial tubes, one on each side of the embryo not far from the median plane and on the ventral aspect of the pleuro-pericardial canals. Recently the bilateral endothelial tubes have been traced to the stage of angioblastic cords, which have also been described as lying between the mesoderm and the entoderm. Spaces soon make their appearance in the vascular masses, and when these coalesce, two endothelial tubes are therefore formed, one on each side of the embryo, ventral to the pleuro-pericardial channels. In the ferret the coalescence of the vascular endothelium is to produce one endothelial tube (the "primary" union of the heart rudiments) lying across the median plane

caudo-ventral

caudo-ventral to the pleuro-pericardial cavity (Stage IIa. Figs. 6a. b. c.). This occurs, it should be noted, at a time before the formation of the fore-gut has made its appearance. When this "primary" endothelial tube is traced laterally, it is found to communicate with the two vitelline veins (Fig. 6a.). A search through the literature on the early stages of development of the mammalian heart has failed to discover any account of this "primary" union of the heart rudiments. Miss Parker, in the course of her investigation into the early stages in the development of Marsupials, notices the early or "primary" union of the heart rudiments across the median plane in Stage II Dasyurus viverrinus (8.5mm.); its significance, however, has not been explained by her.

That the "primary" union of the heart rudiments to form a single endothelial tube is not a singular occurrence due to any abnormal or pathological conditions, and that this stage of development of the heart is an important one is proved by the fact that a similar phenomenon repeats itself in another ferret embryo (Stage IIb, Figs. 11a.b. and 12a.b.c.), in which the heart and the pleuro-pericardial cavity exhibit features similar to those observed in the Stage IIa. ferret embryo. It has to be emphasized once more that the "primary" union of the heart

rudiments

rudiments across the median plane as a transverse vascular channel lying caudo-ventral to the pleuro-pericardial cavity occurs at a period before any indication of the head fold or the formation of the fore-gut can be detected.

The next stage of development of the heart in the ferret embryo is represented by the 2.3mm. embryo (Stage III.). In this specimen, which is obviously slightly older than the Stage IIa. and b., the medial part of the "primary" endothelial tube shows indications of splitting into two lateral endothelial tubes, for two non-vascular loculi divide its central portion incompletely into a cranial and a caudal portion (Fig. 18). Apparently the destruction of the central part of the rudimentary transverse heart ("primary" union) proceeds still further as development goes on, until it is completely separated into a right and left halves; for in the Stage IV ferret embryo the heart rudiments are represented there by two separate longitudinal endothelial tubes lying side by side close together (Fig. 27.). It is again to be noted that the head fold and the fore-gut of this ferret embryo (Stage III) have already begun to develop, since at the cranial end two vessels, one on each side of the median plane, run cranially from the heart rudiment. They arch round the cranial end of the fore-gut and

form

form the first pair of aortic arches, which terminate dorsally in the corresponding dorsal aortae (Fig.18).

In the comparison of this stage of development of the heart in the ferret with that of other mammals of a similar stage of development, the Stage III Perameles nasuta (1.S.) of Miss Parker may be cited; the total length of her specimen, after partial flattening under cover glass, from the anterior margin of the brain-plate to the hinder extremity of the primitive streak is, according to Miss Parker, 7.5mm. In her specimen two endothelial tubes are depicted lying side by side in the pleuro-pericardial cavity. Cranially each endothelial tube becomes continuous with the ventral aorta, which runs underneath the ventral surface of the fore-gut. As the cranial extremity of the fore-gut is reached, the ventral aorta bends dorsally round the blind end of the fore-gut and communicates with the dorsal aorta. In this way the first aorta arch is formed. Whilst still in the pleuro-pericardial cavity the heart tube is described as giving off a lateral branch which is presumably the second aortic arch. What is most striking and interesting in this embryo is that, "In the median space between the anterior ends of the endothelial heart tubes, are a number of scattered angioblast cells lying between the splanchnic

mesoderm

mesoderm and the entoderm". Miss Parker believes that these cells possibly represent the primordia of the capillaries and afford an instance of the origin of angioblast cells from the splanchnic mesoderm after the establishment of the definite endothelial heart tubes. Judging from what has been observed in the ferret embryo of Stage III, it is perhaps more correct to interpret these angioblast cells as being the remains of the once "primary" union of the heart rudiments. This "primary" union is well developed in the ferret embryo of Stage II a. and b. and also in the Stage II Dasyurus viverrinus (8.5mm. A) of Miss Parker. The explanation for the breaking up of the "primary" union of the heart rudiments to form two separate endothelial tubes is, however, not apparent in the light of our present knowledge of the development of the mammalian heart. It is conceivable that partly as a result of the development of the fore-gut cranioward, in the manner pointed out by Robinson(02), and partly also due to the rotatory movement of the heart rudiments from a position ventral to the pleuro-pericardial cavity to a position dorsal to it round a transverse axis, the cross channel, that is, the "primary" union of the heart rudiments is, at first, put on the stretch, and finally separated into two endothelial tubes lying side by side, each of which becomes continuous with

its

corresponding dorsal aorta through the conus arteriosus and the first aorta arch. In the ferret embryo of Stage III (Fig. 18) the connection between the heart and the dorsal aorta has already established itself before the heart, after its "primary" union, has again completely separated into two halves. In the case of the Stage II Dasyurus of Miss Parker, the "primary" union of the heart rudiments is described as giving rise to the first aortic arch, which follows the antero-lateral margin of the gut almost to the median plane and there becomes continuous with the corresponding dorsal aorta. But what has been taken for the first aortic arch by Miss Parker may, after all, prove to be the plexus between the dorsal aorta and the vitelline vein, which has been observed by Bremer(12) in the 3.4mm. rabbit embryo.

It will be noticed then there is evidence to show that, at a period before the formation of the fore-gut, the first rudiment of the heart in the ferret is single and is situated in the median plane of the embryo, caudo-ventral to the pleuro-pericardial cavity, in the form of a transverse endothelial tube which is destitute of any blood cells. Laterally it is in direct communication with the two vitelline veins, one on each side of the embryo. The demarca-

tion

demarcation between the heart rudiment and the vitelline veins is purely a matter of personal interpretation, there being no distinctive characters with which to map out the former from the latter. It is possible to regard that portion of the transverse connection of the two vitelline veins, which lies within the cross pleuro-pericardial cavity, as being the "primary" single heart tube. But exactly how this "primary" heart rudiment is formed it is impossible to make any definite statement. The contention of His(00) does not yet fully account for the origin of this "primary" union of the two vitelline veins, for, according to His, two endothelial tubes are directly formed from the ingrowth of the two vitelline veins which, by a further growth, communicate with the two dorsal aortae. Possibly the "primary" union of the heart rudiments is the result of an ingrowth from the vitelline veins which, instead of linking up each with its corresponding dorsal aorta, as His would have imagined, have grown across the median plane. In this way the U-shaped vitelline system is, at least for a time, not connected with the longitudinal aortic system cranially. This is exactly what is seen in the ferret embryo of Stage IIa. (Fig. 6a). It is also possible, and perhaps more probable, that the "primary" union arises in situ and is afterwards joined to the two vitelline veins.

Of late years the weight of evidence seems to indicate that the formation of intra-embryonic blood vessels in situ is much more extensive and important than has hitherto been supposed (Stockard(15), Hahn (09), Miller and McWhorter(14), Reagan(15) and others).

From whichever point of view the development of the heart is looked upon, it is clear that in the ferret the first rudiment of the heart appears as a cross channel situated ventro-caudally to the pleuro-pericardial cavity, and is connected only with the venous system, which is composed of the two vitelline veins laterally. The arterial system, that is, the dorsal aortae, remains, at least for a time, distinct and unconnected with the heart rudiment. No explanation can at present be offered as to why the heart rudiment, when first represented, should only be united with the venous circulation. It is evident that further light in this field of investigation is required before a solution can be obtained.

Quite recently Professors Robinson and Gibson (16), in their description of a reconstruction model of a horse embryo twenty-one days old, mention that "The allantoic blood vessels consist of a number of dilated capillaries which form a coarse net-work on each side. Each lateral net-work receives two branches from the caudal end of the dorsal aorta of the same side, and it terminates, at the caudal end

of

of the allantoic mass, in a terminal transverse sinus from which the umbilical veins take their origin. But, in addition to the connection with both umbilical veins through the terminal sinus, each vascular net-work also communicates directly with the umbilical vein of the same side". The terminal transverse sinus at the caudal end of the allantoic mass of the horse and the transverse heart rudiment situated in the cranial end of the ferret embryo may together represent at one time a portion of an original complete ring of circulation.

The next phase of development of the heart is represented by the separation of the "primary" heart rudiment into two distinct endothelial tubes lying closely together, one on each side of the embryo, not far from the median plane (Stage IV.). Coinciding with the development of the heart, the head fold appears, and as the formation of the fore-gut proceeds, the heart rudiment suffers a reversion with regard to its relation with the pericardium, for it is seen that when the fore-gut is formed the heart is found on the dorsal aspect of the pleuro-pericardial cavity and is attached to the ventral surface of the fore-gut.

Each tube is covered by the splanchnic wall of the pleuro-pericardial cavity on its lateral, ventral and medial surfaces. Dorsally they are connected

with

with the ventral aspect of the fore-gut by the reflection of the wall of the pleuro-pericardial cavity. Ventrally the pleuro-pericardial cavity passes from side to side (Fig. 27.). It is to be noted that the two tubes lie far apart from each other cranially and caudally where they emerge from the pleuro-pericardial cavity. The distance between them is greater caudally (Fig. 26) than cranially (Figs. 27 and 28d). It is obvious that at a stage further, when the two heart rudiments are brought together to form one endothelial tube, it is connected only with the ventral surface of the fore-gut by a dorsal mesocardium and no ventral mesocardium can possibly be developed.

Cranially each endothelial tube can be traced to its corresponding dorsal aorta through the first aortic arch, and, caudally each tube communicates with its corresponding vitelline vein in the region of the septum transversum (Figs. 21. 25. and 27).

It is generally held that the rate of growth of the two endothelial tubes exceeds that of the pleuro-pericardial cavity. Consequently as the result of the different rate of growth the two tubes instead of having a straight appearance, are thrown into loops before the "secondary" union takes place.

Miss Parker(15) thinks, however, that at this stage of development the pericardium grows rapidly

in

in length and decreases in width so that the heart tubes are brought together by longitudinal stretching of that part of the pericardial wall which lies between them. In the ferret it is clear that this is not the case; the two endothelial tubes seem to grow more rapidly in length than the pericardium, with the result that loops are formed with constrictions here and there to mark out the different subdivisions of the heart (atrium, ventricle, bulbus etc.) before any fusion of the two tubes takes place (Stage IV. Fig. 27.), these subdivisions appear to remain even when the two endothelial tubes have partially united (Stage V. Fig. 31). The appearance of these loops in the endothelial tubes speaks against the theory of stretching advanced by Miss Parker. For if it were true, the result of any stretching of that part of the pleuro-pericardial wall which lies between the medial borders of the two endothelial tubes, would, in the first instance, be the undoing of the loops, or the prevention of their formation, before any approximation of these tubes could be affected.

In the ferret (Stage IV) what has really happened is this, that as the result of the rapid growth, the two endothelial tubes are thrown into loops, and as the result of a further growth of the two tubes medially towards each other, the part of the pleuro-pericardial wall which lies between them is pushed ventrally.

ventrally. When fusion of the two endothelial tubes takes place, the unpaired heart is, therefore, attached only to the ventral wall of the fore-gut by the dorsal mesocardium. Ventrally the splanchnic wall of the pleuro-pericardial cavity passes from side to side across the ventral aspect of the fused heart tube, there being no fusion of this part of the pleuro-pericardial wall, as supposed by Miss Parker.

Asymmetry of the heart tubes, either before or after fusion takes place, has been noted in the ferret embryos (Stages IV. and V. Figs 27. and 31) as in the Stage IV Perameles nasuta and also, in a measure, in the Stage V Perameles obesula (10.viii.03) of Miss Parker and likewise in the 5.7mm. dog embryo of Bonnet(01). There seems to be a tendency for the two endothelial tubes, even before fusion, to curve and to be shifted distinctly as a whole to the right side with the concavity of the bend facing the left. In Stage V (Fig. 31) the fusion of the two endothelial tubes in the ferret takes place in the ventricular region, and the fused part lies clearly on the right side of the median plane. The fact of this occurrence contradicts, in a very convincing manner, the theory of stretching of the pleuro-pericardial wall, for the fused portion of the two endothelial tubes should occupy the median plane of the embryo if the two tubes were really brought together by the uniform

stretching

stretching of that part of the splanchnic wall of the pleuro-pericardial cavity which lies between them. There is no evidence to prove that the fusion of the two tubes, occurring on the right side, may be due to an uneven stretching of the dorsal wall of the pleuro-pericardial cavity.

In the Stage IV Perameles nasuta (2.P) of Miss Parker the description of the heart in the text does not seem to agree with her illustration (Plate 1. fig. 5). In the text it is stated: "They (the two endothelial tubes) have fused at their cephalic extremity, the fused portion extending through some eighteen sections and representing the most closely approximating portions of the endothelial tubes.....From it is derived the bulbus (conus) arteriosus..... Posterior to this fused portion, the endothelial tubes lie close together but unfused for a considerable portion of their length and then diverge widely and pass into vitelline veins". In the illustration, on the other hand, it appears that the fused portion represents really more than the conus arteriosus. Possibly the ventricles also have fused across, because in the figure it shows that at least the cranial half, if not more, of the two endothelial tubes have fused. If this is the case it is difficult to explain why in the next stage of development (Stage V. Perameles

Perameles obesula (10.viii.03) of Miss Parker) the bulbus is the only portion of the heart in which the endothelial tubes have actually fused, and the ventricles have become once more separated.

In the text and the figures illustrating Stage IV. Perameles nasuta (2.P) and Stage V Perameles obesula (10.viii.03) of Miss Parker, there are no data to be found upon which the magnification and length of the embryos in question can be gauged. But as far as one can judge from the illustrations alone, the figures of Stage V. Perameles obesula are undoubtedly of a higher magnification although probably taken from an embryo of a greater length than that of the Stage IV Perameles nasuta. If it is so, an explanation is needed to account for the decrease in length of the fused portion of the two endothelial tubes seen in Stage V. It is unfortunate that these important data should have been omitted by Miss Parker in her paper, for their absence diminishes the value of her communication when an attempt is made to use it for the purpose of solving the points under consideration.

Hitherto the heart tube with its time-honoured S-shaped character has been the subject of much dissertation. The literature on the development of the S-shaped heart rudiment is so voluminous and confusing that only a brief summary on this subject

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can be given. All writers have laid great stress on the subsequent development of the fused endothelial tube which is now covered with its myocardial coat. It is generally believed that the next phase of development of the fused heart tube, consisting of an inner endothelial tube and an outer myocardial covering, is the acquisition of the well-known S-shaped form. The caudal portion of the single heart tube grows cranio-dorsally and to the left to form the atrial limb, and the cranial portion grows caudo-ventrally and to the right to form the ventricular limb. In the Stage IV ferret embryo model (Fig. 22) it is to be remembered that with the ventral wall of the pleuro-pericardial cavity removed the muscular heart tube has been seen to be partially divided in the caudal but larger portion of its extent into a right and left segment by a cranio-caudal sulcus. On each lateral half there are also several transverse sulci, but the significance of these various sulci is rather obscure as the segments marked out by them do not correspond, in any way, with the primary divisions of the heart tube, although at first sight, it appears that the left segment is apparently atrial, and the right, ventricular. The fact that this appearance is deceptive becomes at once obvious when a portion of the muscular wall of

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the heart tube is removed (Fig. 27), for it is then seen that within the lumen of the muscular tube and separated from its walls by a more or less thick mass of loose mesodermic tissue, lie two endothelial tubes which are quite separate from one another in the whole of their extent. In the reconstruction (Fig. 27) the muscular coat of the heart rudiment has been removed entirely on the left side and partly on the right side. In this way the left endothelial tube is, therefore, fully exposed to view on its ventral and lateral aspects, and it exhibits clearly a very definite indication of separation into divisions which appear to suggest, in caudo-cranial succession, the positions of the sinus venosus, the sino-atrial canal, the atrium, the atrio-ventricular canal, the ventricle and bulbus cordis.

The right endothelial tube, which has not been fully exposed in the model, appears to present similar dilatations and constrictions, the outlines of which can be followed to a certain extent through the muscular covering, but since, in the model, it is still covered in parts by muscular substance, the exact details cannot be worked out at present and therefore no positive statement regarding it can be put forward. It is, however, more dorsally situated than the left tube. It is to be observed that the whole heart is bent slightly towards the right side

in

in the ventricular region.

At this stage of development of the ferret embryo (Stage IV) it is evident that the external configuration of the muscular wall of the heart does not furnish a true index to the condition of development of the enclosed endothelial tubes, for it has been remarked upon that although the muscular coat of the heart rudiment may have acquired the familiar S-shaped appearance, yet the two endothelial tubes have not begun to fuse. Moreover, the segments exhibited in the muscular tube do not correspond, in the least, with the dilatations and constrictions of the underlying endothelial tubes. Furthermore, in the dog, it has been pointed by Bonnet, and in Marsupials by Miss Parker that primary divisions of the endothelial tubes into sinus venosus, atrium and ventricle occur before they have completely fused to form a single tube. Similar divisions of the heart tubes are present also in the Stage IV ferret embryo (vide supra). These facts therefore show that the primary divisions of the heart rudiment take place in the endothelial tubes at a time when no fusion can be detected, and that the convolutions of the S-shaped muscular coat of the heart do not necessarily represent the different segments of the underlying endothelial tubes.

In connection with the fusion of the two endothelial

endothelial tubes there is one more point which requires attention. Many investigators have noted the asymmetry of the heart rudiments at this period of development. Deviation of the two heart tubes to the right, before and after fusion, has also been observed in the mammalian heart. In the ferret these features are present. The significance of the asymmetry and deviation of the two endothelial tubes have, so far, not been fully explained by those who have made these observations. It has been noted that the ventricular portion of the heart is the first to fuse, and is situated on the right side of the embryo (Stage V. Fig. 31). The fused portion in fact represents the junction between the ventricular and the atrial limbs of the future S-shaped heart tube. As fusion proceeds cranially and caudally, more parts of the heart tubes are taken in to form the two limbs of the S-shaped heart rudiment until the two tubes are completely fused. The result of this fusion is to produce a ventricular limb which is situated ventrally and to the right and an atrial limb which is directed dorsally and to the left.

It should also be remembered that in the space between the myocardium and the endothelium of the heart tubes there is a more or less thick mass of loose mesodermic tissue which separates not only the former from the latter but also the two endothelial

tubes

tubes from one another before their fusion (Stage IV. Figs. 27. and 28a.b.c.). This mass of loose tissue, though playing an important rôle in the establishment of the sino-ventricular bundle(Mall(12)) in the subsequent development of the heart, is essentially a passive element at this stage of development. It permits, on the one hand, the growing endothelial tubes to assume their various bendings, constrictions and dilatations independently, and adapts itself, on the other hand, to the characteristic curling of the muscular tube into an S-shaped appearance. This point is clearly shown in the Stage IV. ferret embryo in which the two endothelial tubes have, as already noted, differentiated into their various divisions in advance of their fusion (Fig. 27), whilst the muscular wall of the heart has seemingly acquired the familiar S-shaped form (Fig. 22). With the ventral wall of the pericardium of this embryo removed (Fig. 22) it has already been noticed that the ventral aspect of the myocardial covering of the heart is separated in the caudal but larger portion of its extent into a right and left segment by a cranio-caudal sulcus. Cranially these two segments are confluent with each other over a narrow transverse area. The two segments together with this transverse area present an S-shaped appearance. At first sight it appears that the left segment is atrial, the right, ventricular,

ventricular, and the transverse connecting portion, the atrio-ventricular canal. But with the left portion of the muscular wall of the heart removed (Fig. 27), what appears to be the atrial bulge of the muscular coat is, in reality, caused by the underlying atrial portion of the left endothelial tube only; the atrio-ventricular canal of the muscular coat is caused by the unfused right and left ventricular portions of the two endothelial tubes which are closely applied together in this region; and the ventricular segment of the muscular wall is due to the atrial portion of the right endothelial tube (Compare Figs. 22 and 27). It is obvious, therefore, that owing to the interposition of this more or less thick mass of mesodermic tissue, the endothelial tubes do not follow, in a faithful manner, the various curvatures and bulges of the myocardial covering of the heart, and it would be a mistake to try to determine, at this stage of development, the true nature of the two endothelial tubes by a simple examination of the condition and shape of the muscular coat, without ascertaining, at the same time, the various features of the underlying endothelial tubes and comparing these with those exhibited by the muscular covering.

It may therefore be concluded that, at least, from the first appearance of the paired heart rudiments as two endothelial tubes to the time of their "secondary"

"secondary" fusion, the myocardium has little or no common relationship with the underlying endothelium; that the two structures are quite independent of each other, as far as their individual growth is concerned; that the various constrictions and dilatations of the two endothelial tubes have a definite significance; that these constrictions and dilatations fore-shadow the site and limits of the future sinus venosus, sino-atrial canal, atrium, atrio-ventricular canal, ventricle and bulbus cordis; and that the muscular tube comes into conformity only at a later period.

SUMMARY AND CONCLUSIONS.

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A. THE BLOOD CELLS AND VESSELS.

Hitherto it has been the general belief that blood cells and vascular endothelium in mammals are derived from a common origin which, according to some, is mesodermic, and others, entodermic, and that, whichever may be the source of origin, the vascular rudiment appears, at first, as angioblastic cells lying between the mesoderm and entoderm. It has been claimed also that the peripheral part of the angioblast soon resolves itself into an uninterrupted network of endothelium, and the central part into clusters of blood cells. It has been stated further that the endothelium so formed is capable of giving rise to new blood cells.

The facts revealed by the study of the early stages in the development of the ferret point to the conclusion that, whilst blood cells and vascular endothelium are closely related to each other and are found invariably between the mesoderm and entoderm, but there is evidence to show that, in the ferret, the origins of these two vascular elements are separate and distinct - the blood cells arising from the entoderm

entoderm and the vascular endothelium from the mesoderm.

Blood cells develop first extra-embryonically in the area vasculosa in the form of clusters of spheroidal cells which are provided with large and round nuclei and with a comparatively small amount of protoplasm. These are for the most part found adherent to the entoderm in the neighbourhood of their origin before they are engulfed by the endothelium and are, in most instances, identical in structure with the entodermal cells where the contact is intimate.

On the other hand, the cells which form the endothelial rudiment are mesodermic in origin and are, without exceptions, spindle or flattened in shape. They are generally connected with one another by long slender protoplasmic processes, the result of which is to form an uninterrupted net-work of endothelium. This net-work is capable of extension by buddings which grow either into the embryo or on to the yolk-sac. The blood cells are next engulfed by the vascular endothelium which grows round them, and in this way they are taken into the circulation.

In the ferret embryo the blood cells and vascular endothelium are not interchangeable, that is to say, the blood cells are incapable of metamorphosis into endothelial cells and the vascular endothelium, once formed,

formed, has not the power to generate any new blood cells.

B. THE INTRA-EMBRYONIC BLOOD VESSELS.

The caudal portion of the dorsal aorta communicates, at a very early stage of development, with the yolk-sac circulation through the vitelline arterial plexus. Cranially the dorsal aorta stops short and remains unconnected with the heart rudiment as long as there is no head fold or fore-gut. This part of the dorsal aorta makes its appearance in association with the development of the head fold and fore-gut.

The conus arteriosus and the first aortic arch develop after the "primary" heart rudiment, the two vitelline veins, and the dorsal aortae are all represented, and at the time when the formation of the head fold and fore-gut has begun.

The vitelline veins are two in number, one on each side of the embryo. They lie for the greater part of their extent ventro-medial to the pleuro-pericardial canals. They grow from the wall of the yolk-sac into the cranial extremity of the embryo. They are, at an early period, united across the median plane to from the "primary" heart rudiment which lies between the pleuro-pericardial cavity dorso-cranially and the bucco-pharyngeal membrane caudally.

A short distance caudal to the "primary" heart rudiment, each vitelline vein sends out off-shoots medially to anastomose with the cranial end of its corresponding dorsal aorta.

C. THE PERICARDIUM AND THE HEART.

Before there is any indication of the head fold or the formation of the fore-gut, the pleuro-pericardial canals have grown across the median plane of the cranial end of the embryo to form the pleuro-pericardial cavity which, in relation with the "primary" heart rudiment, lies cranio-dorsal to it. As the head fold and fore-gut develop by growing cranially, a rotation of the pleuro-pericardial cavity and the heart rudiment round a transverse axis, takes place, with the result that the former occupies a position ventral to the latter and the "primary" heart rudiment is now found ventral to the fore-gut.

In the subsequent stages of development the "primary" heart rudiment divides into two endothelial tubes, each of which is covered laterally, ventrally and medially by the splanchnic wall of the pleuro-pericardial cavity and is attached only dorsally with the ventral aspect of the fore-gut by the reflection of the pleuro-pericardial wall. As a result of the growth of the two endothelial tubes towards the median

plane

plane, the part of the splanchnic wall of the pleuro-pericardial wall which lies between them, is thereby pushed ventrally. When fusion of the two endothelial tubes occurs, the "secondary" heart tube is consequently attached only dorsally to the ventral surface of the fore-gut by the dorsal mesocardium. Ventrally there is no fusion of the pleuro-pericardial wall, and therefore no ventral mesocardium can possibly be developed.

At an early period when the pleuro-pericardial cavity has not yet reversed its position and when there is no indication of the formation of the head fold or the fore-gut, the heart rudiment appears as a transverse blood channel - the "primary" heart rudiment - situated in the median plane ventro-caudal to the pleuro-pericardial cavity and cranial to the bucco-pharyngeal membrane. Laterally the "primary" single heart rudiment communicates with the two vitelline veins, one on each side of the embryo. At this stage of development the cranial extremities of the two dorsal aortae are found, for a considerable distance, caudal to the heart rudiment.

In the subsequent development of the embryo when the head fold and the fore-gut make their appearance by growing cranially, the pleuro-pericardial cavity and the "primary" heart rudiment undergo a rotation round a transverse axis, with the result that

their

their positions are reversed, so that the heart rudiment now lies dorsal to the pleuro-pericardial cavity.

The division of the "primary" single transverse heart rudiment into two longitudinal endothelial tubes is due to the fact that, at this period, the fore-gut grows rapidly cranially in length and ventrally in width, together with the expansion of the pleuro-pericardial cavity in all directions, so that the transverse heart rudiment which lies between these two structures, is at first put on the stretch and is subsequently divided across into two endothelial tubes, a right and a left. Concurrently with the development of the head fold and the fore-gut the two dorsal aortae establish their communications with the "primary" heart rudiment even before the latter is completely separated into two endothelial tubes.

The next phase of development of the heart is the appearance of the two separate endothelial tubes, one on each side of the median plane. Each tube is continuous cranially with the dorsal aorta and caudally with the vitelline vein. As development proceeds they tend to grow towards the median plane and in this way the part of the splanchnic wall of the pleuro-pericardial cavity which lies between them, is pushed ventrally. The two tubes remain in contact, but not fused, with each other for a time. Owing to the fact that the endothelial tubes grow more rapidly than the

pleuro-

pleuro-pericardial cavity, the former are thrown into loops which are separated by constrictions. These dilatations and constrictions indicate in caudo-cranial succession the future sinus venosus, the sino-atrial canal, the atrium, the atrio-ventricular canal, the ventricle and bulbus cordis. The most dependent and approximating parts of the endothelial tubes are the ventricular portions. They incline more to the right side of the embryo and are the first to become fused and to form the "secondary" single heart tube.

The myocardium assumes the familiar S-shaped appearance at a stage when the heart is still in the condition of two separate endothelial tubes. The segments and sulci, appearing on the surface of the myocardial tube, do not correspond, in any way, with the dilatations and constrictions of the two underlying endothelial tubes.

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